

204040" 254T+660

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#6

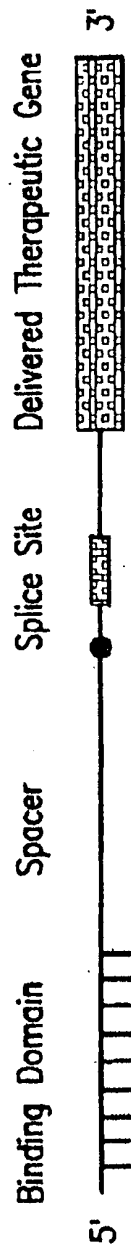
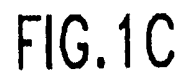


FIG.1A



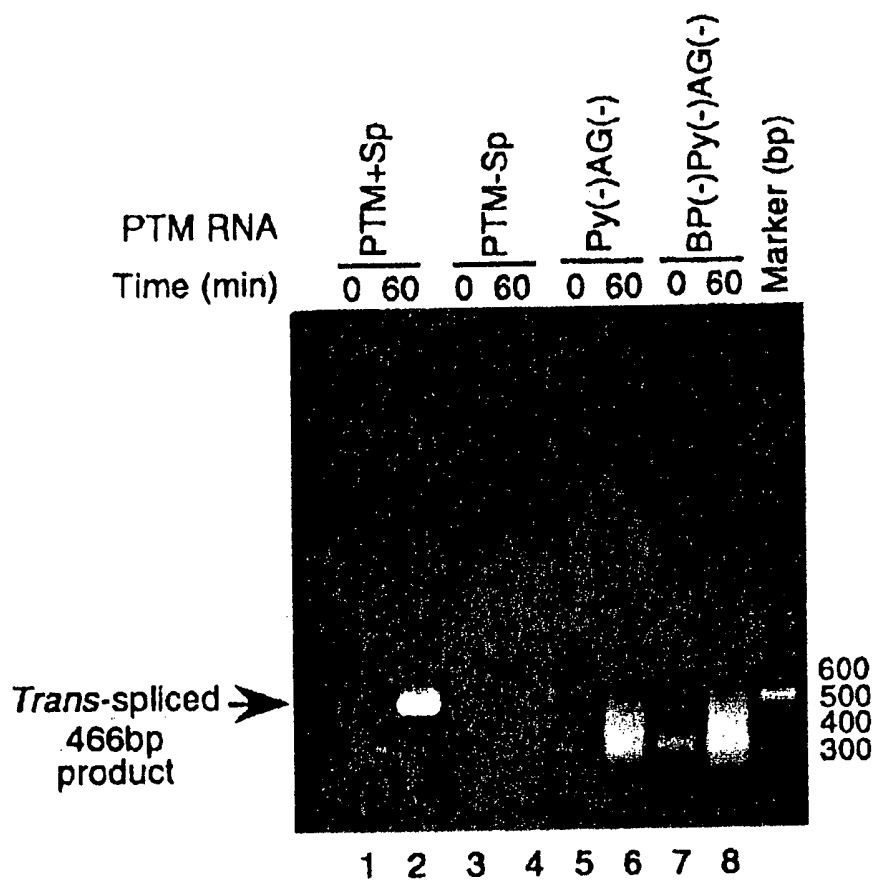


FIG.2A

207070" 264T4650

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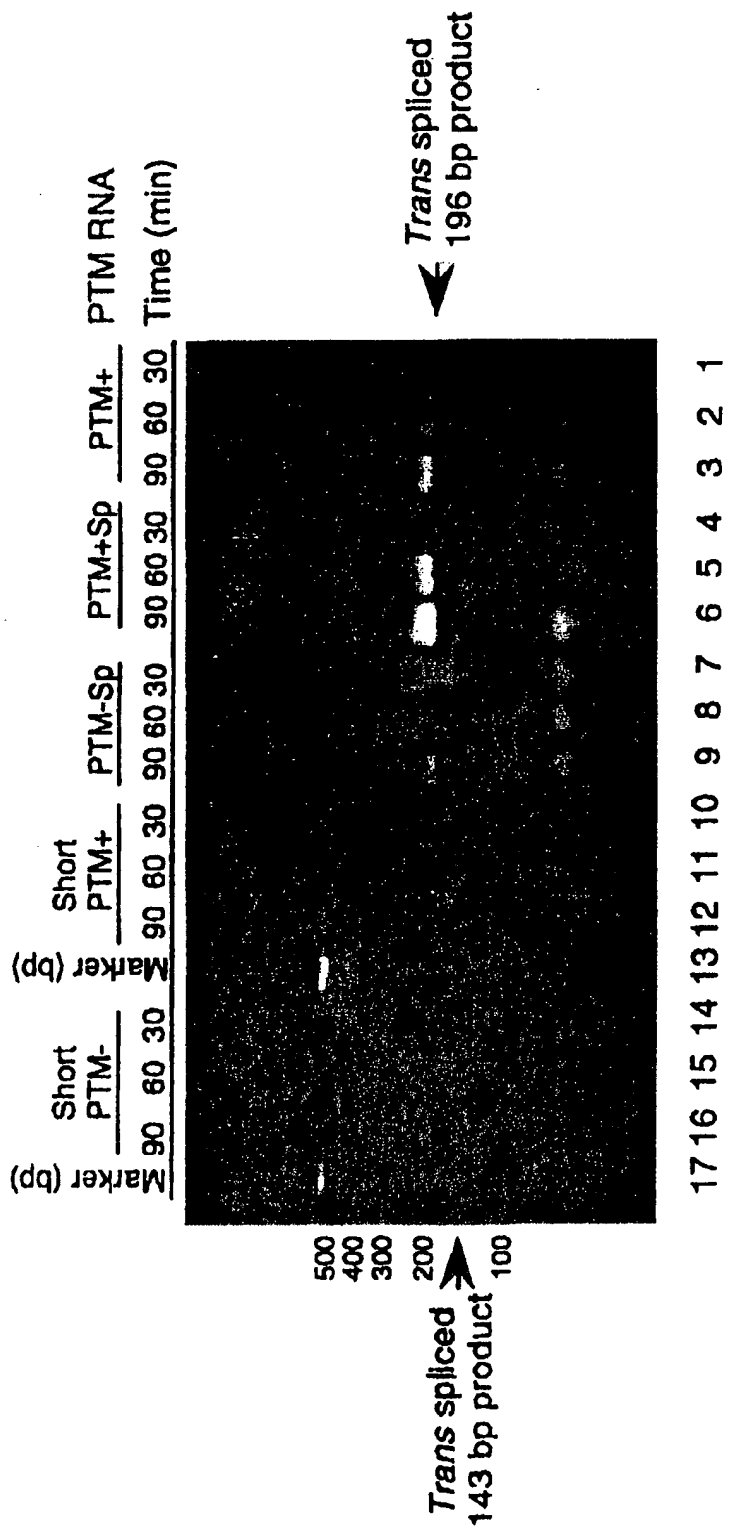


FIG.2B

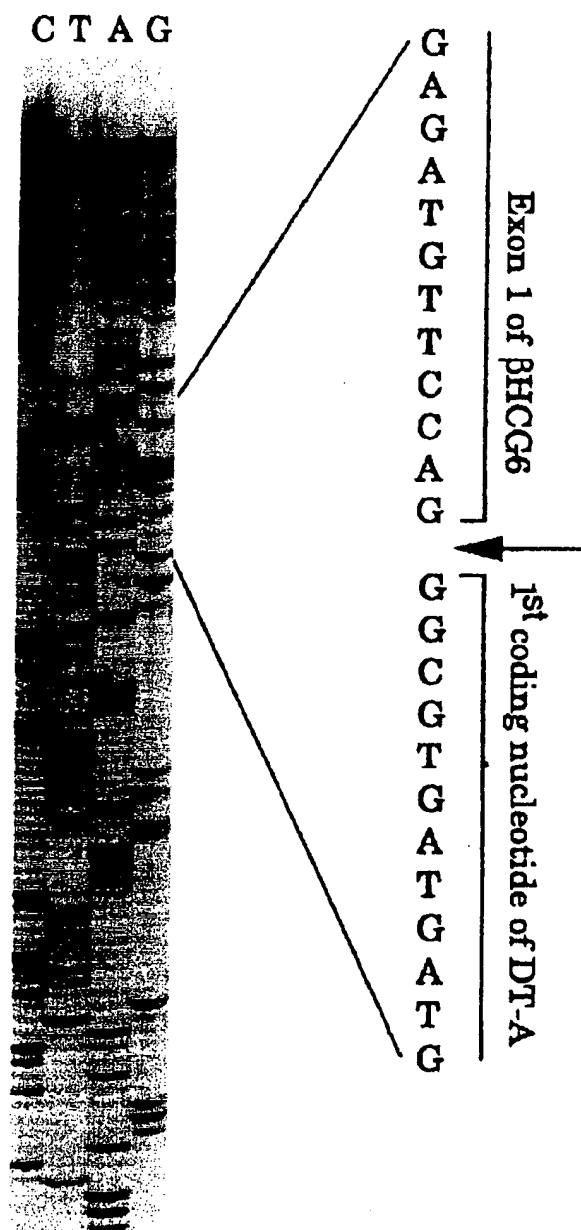
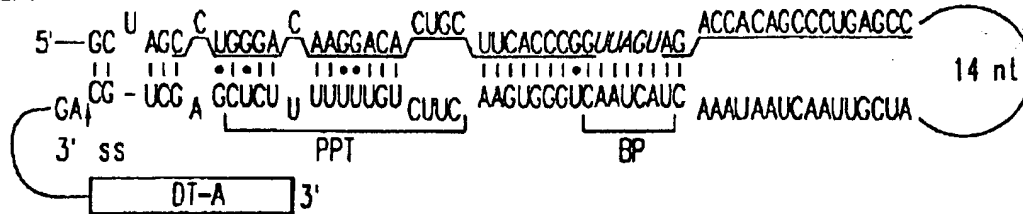


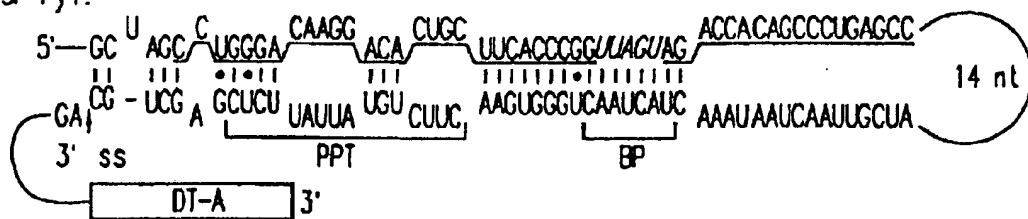
FIG.3

09941492.040402

1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

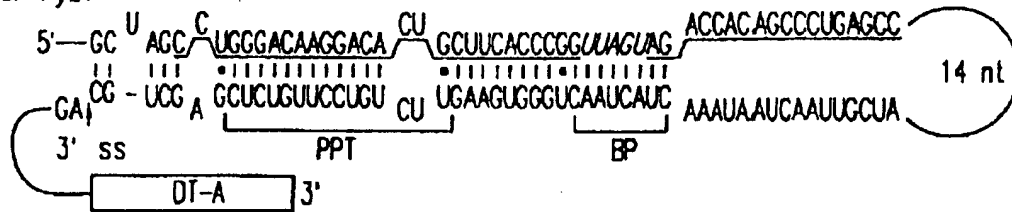


FIG.4A

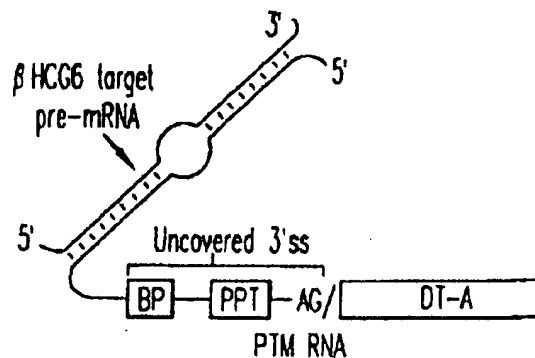


FIG.4B

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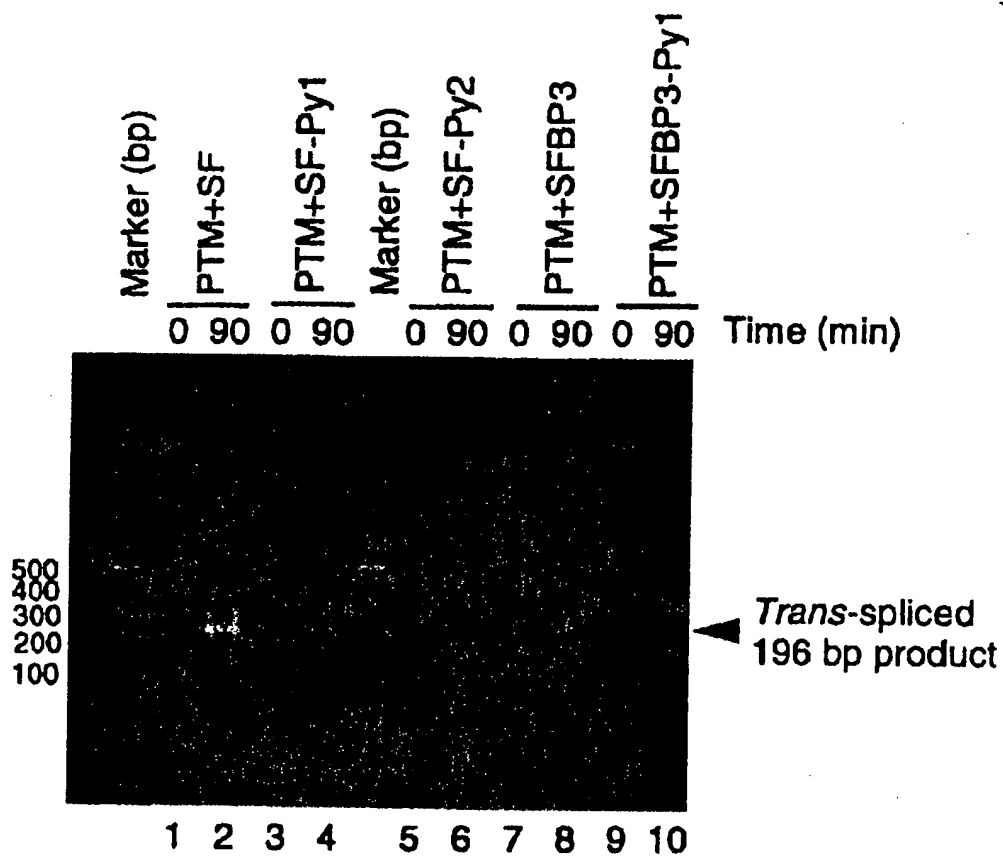


FIG.4C

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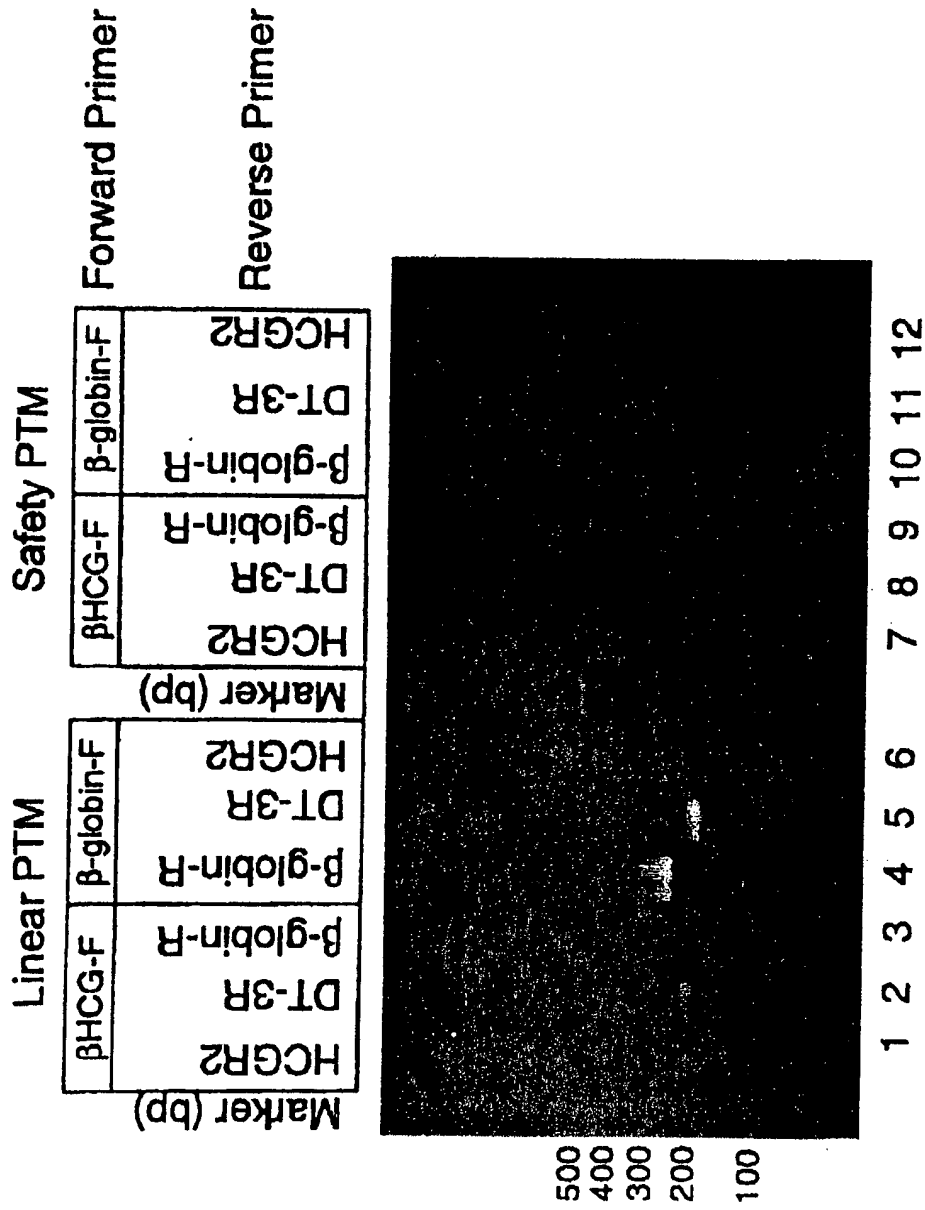


FIG.5

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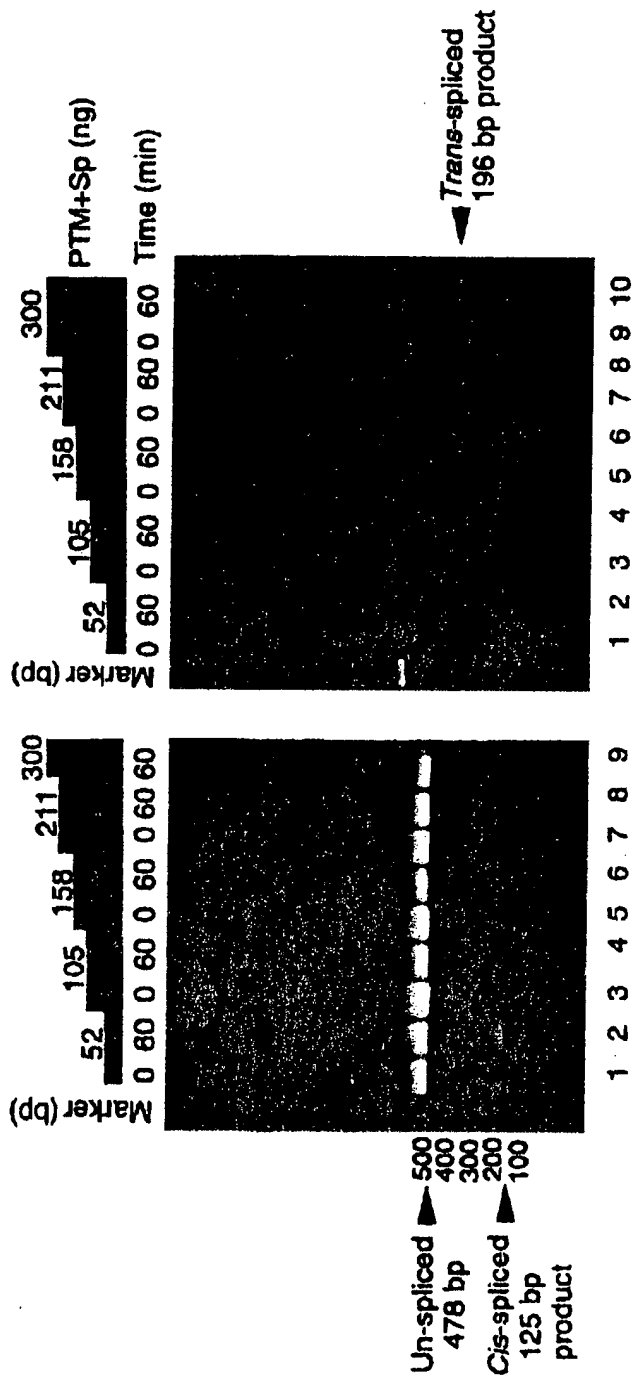


FIG. 6A

FIG. 6B

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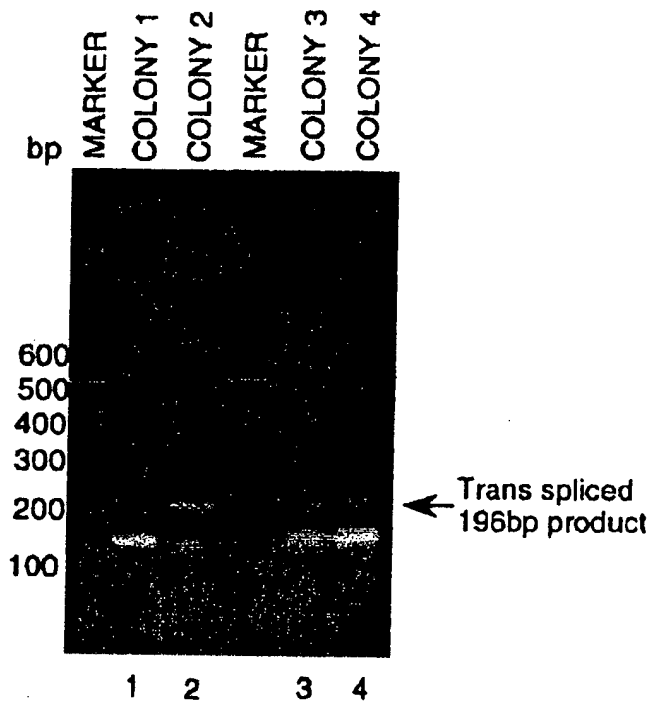


FIG.7A

204040" 2647450

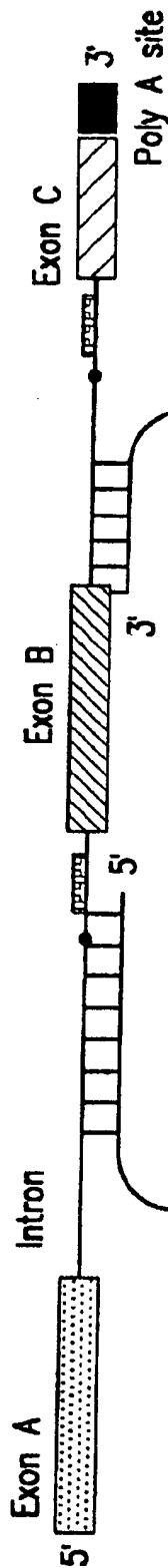
EXON 1 OF β HCG6 †
5'-CAGGGACGCACCAAGGATGGAGATGTTCCAG-GGCGCTGATGATGTTGTT
† 1ST CODING NUCLEOTIDE OF DT-A
GATTCTTCTTAAATCTTTTGTGATGGAAACITTTCTTCTGTTACCAACGGGACTA
AACCTGGTTAIGTAGATTCCATTCAAAA-3'

11 8 91

FIG.7B



Cis-splicing



Double splicing pre-therapeutic RNA

Trans-splicing

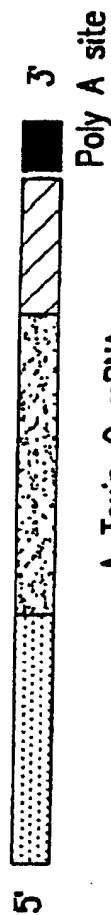
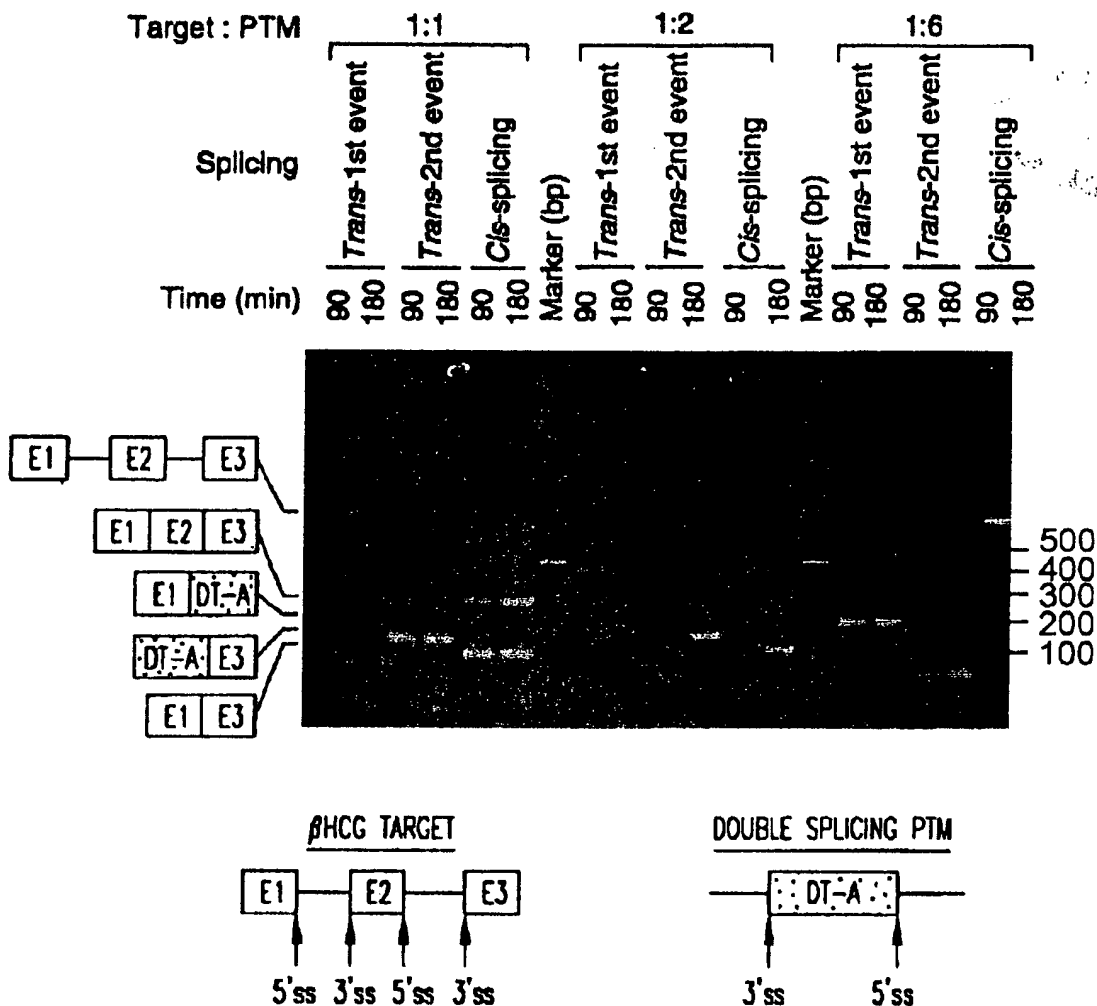


FIG.8A

12 8 91



Cis-spliced products

E1 E2 E3 = NORMAL cis-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. Trans-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. Trans-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

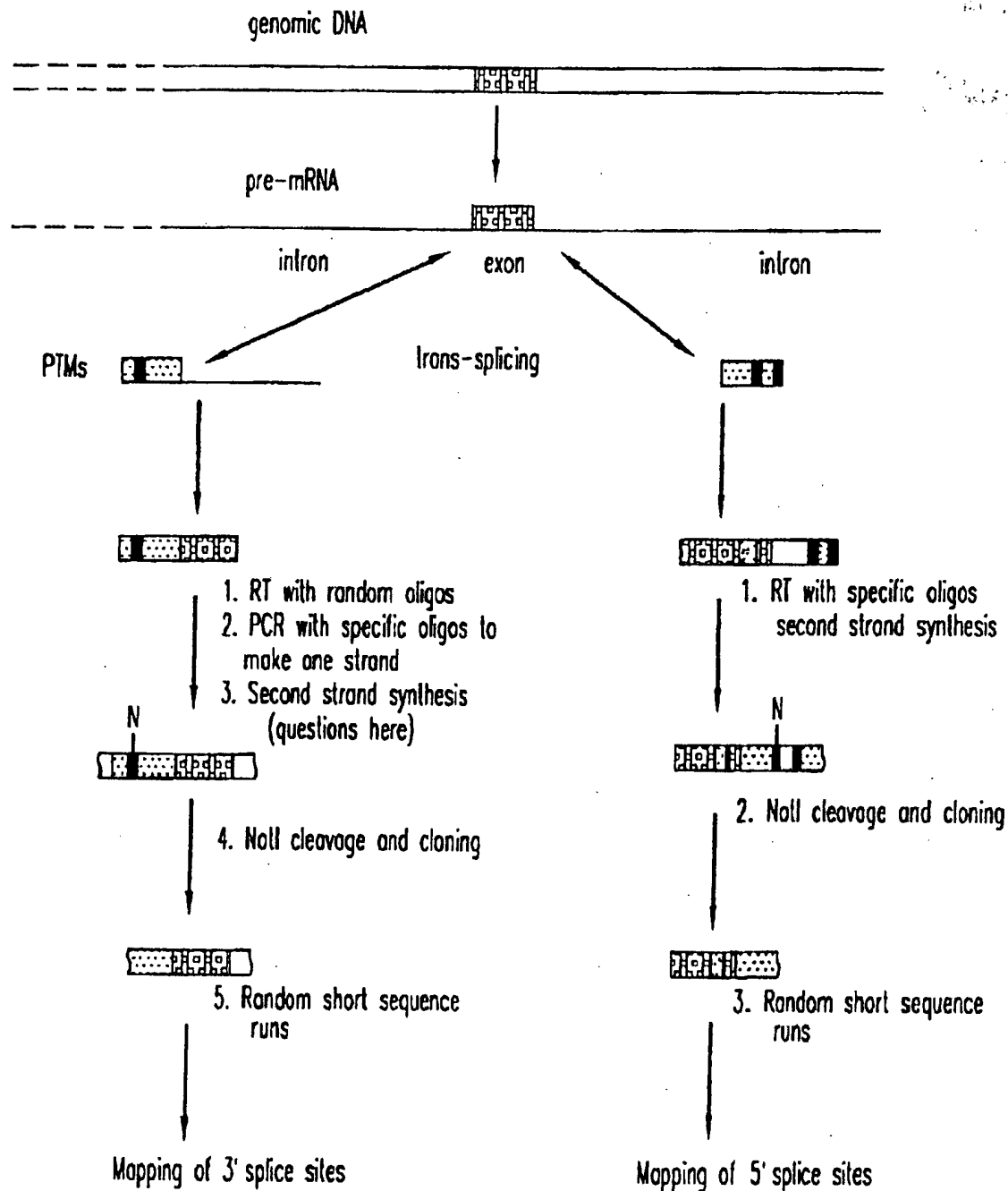


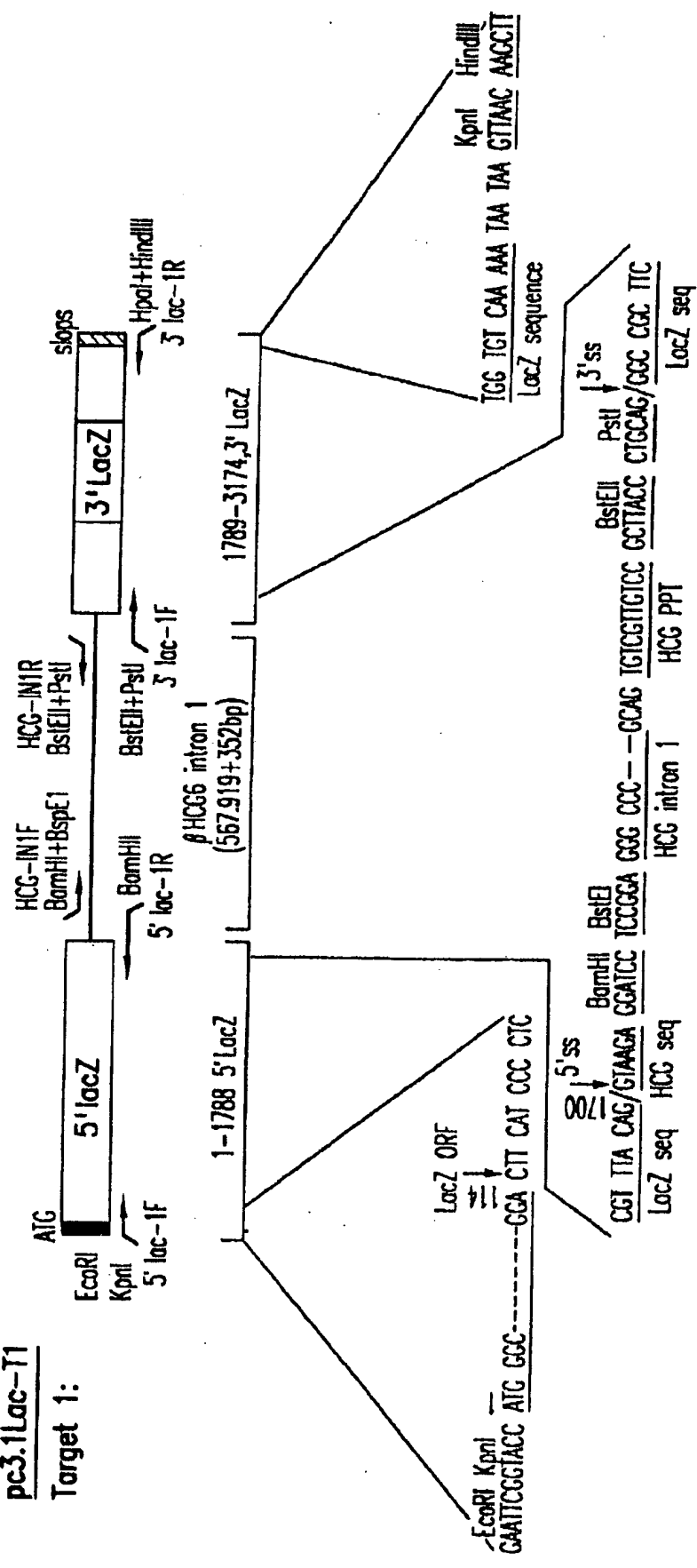
FIG.9

2014040"25474650

204040" 26474650

pc3.1Lac-T1

Target 1:



pc3.1PTM2:

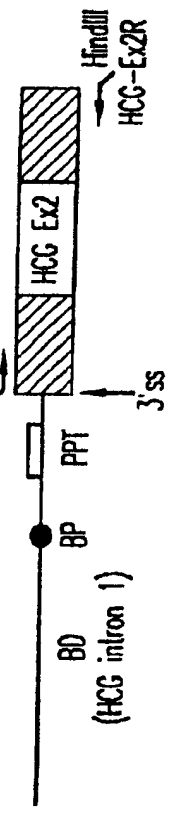


FIG.10A

16 8 91

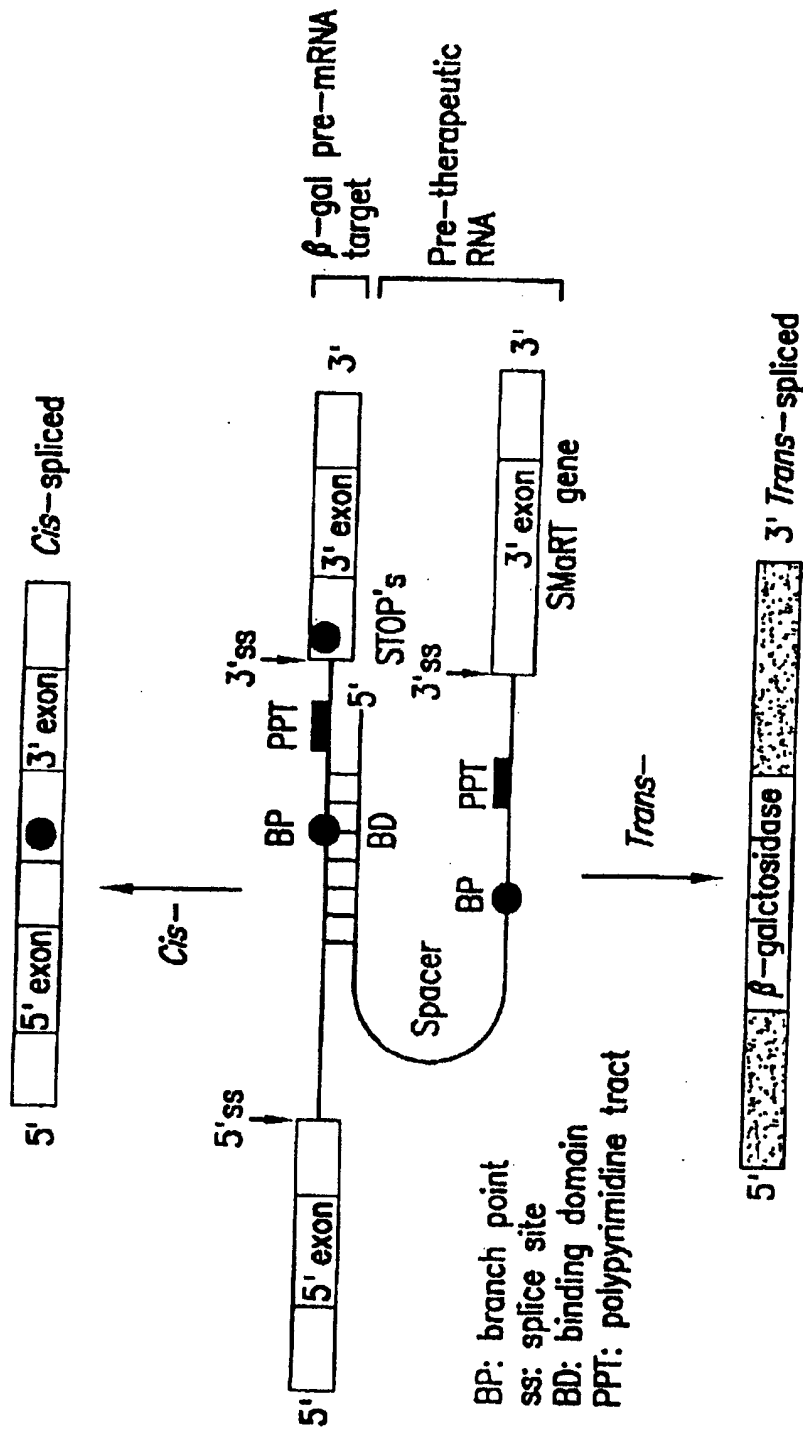


FIG.10B

17 8 91

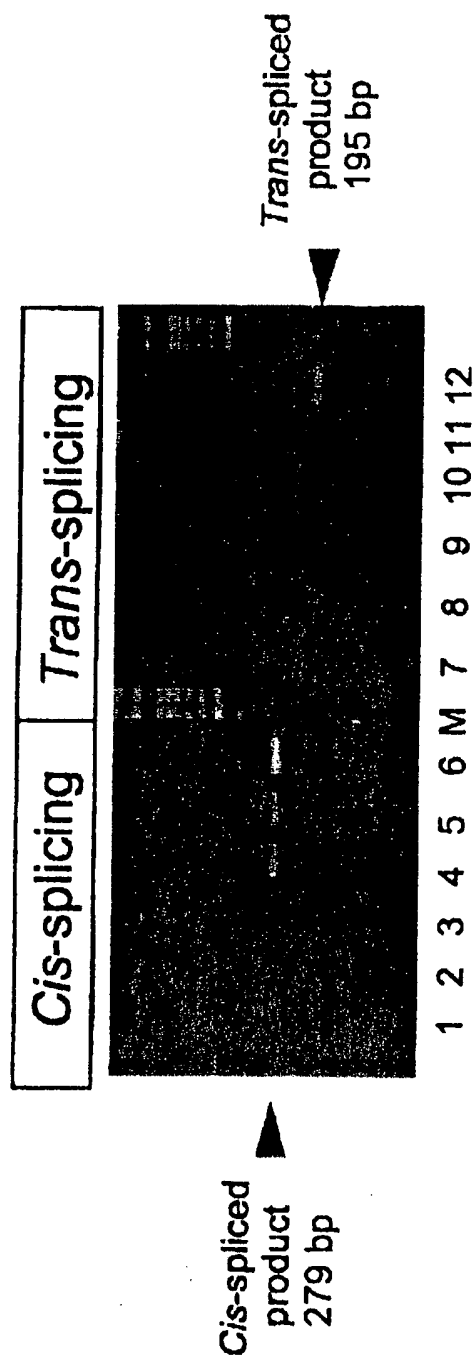


FIG.11A

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204040 25474650

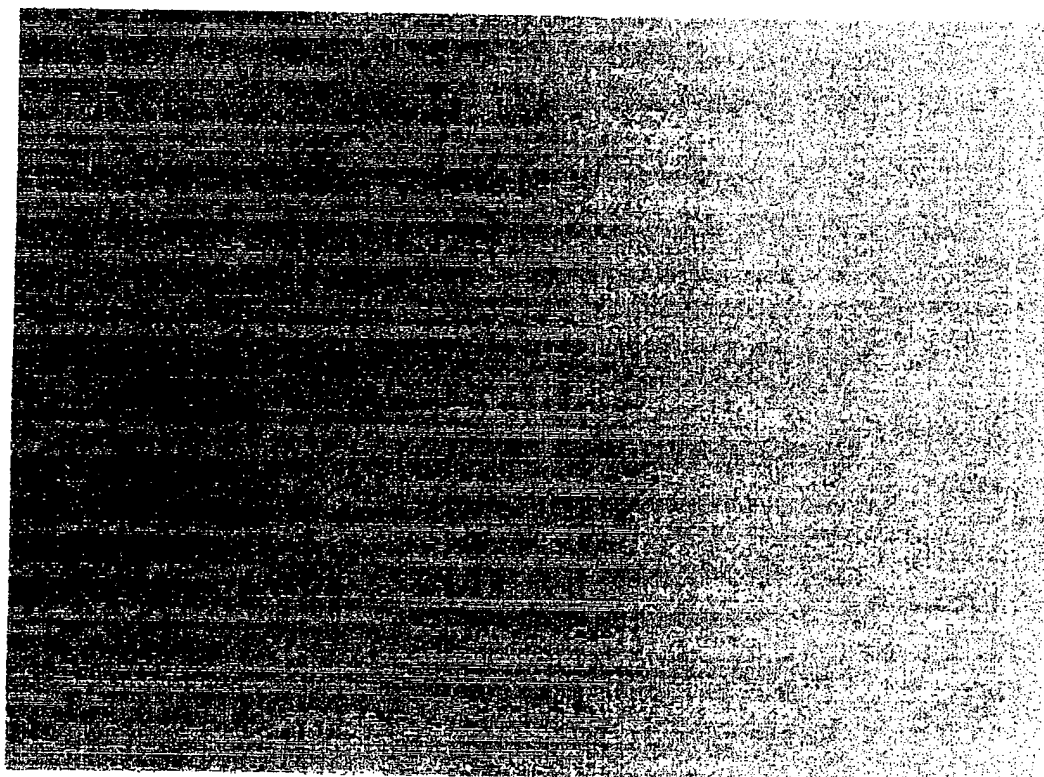


FIG. 11B

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09941492.040402

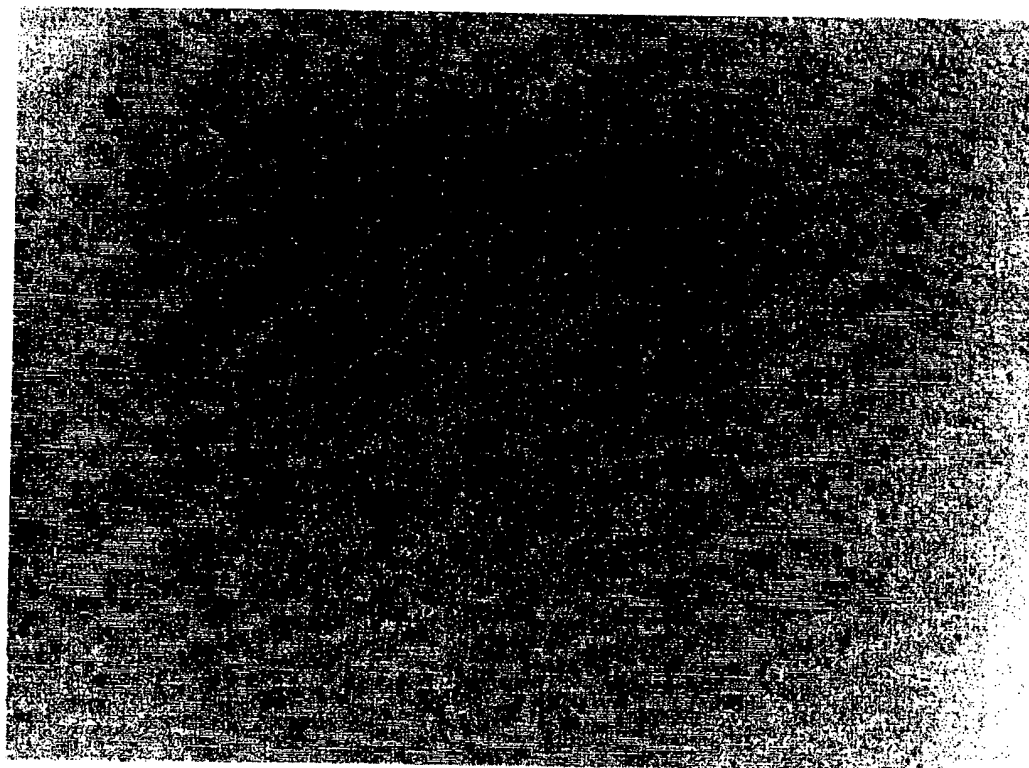


FIG.11C

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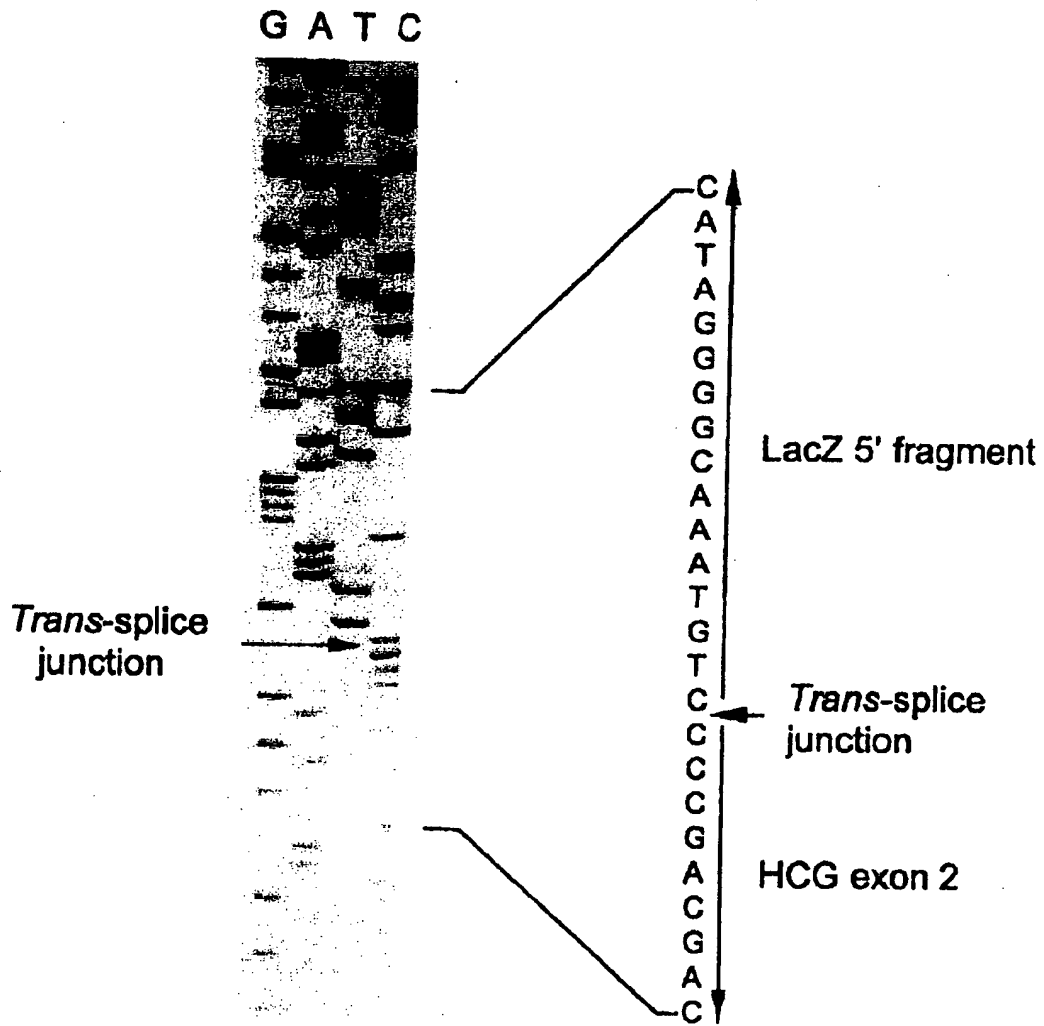


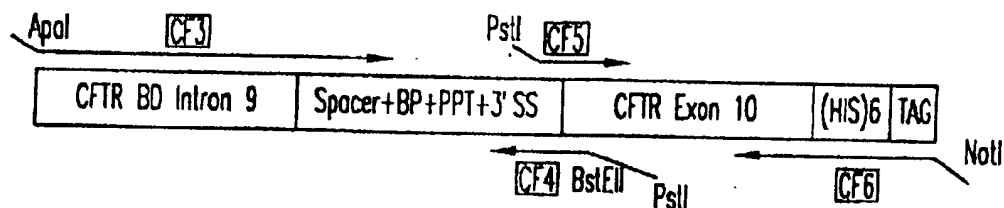
FIG.12A

1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):
 BioLac-TR1
GGCTTTGGCTACCTGGAGAGACGGCCCGCTGATCCTTTGGGAATACGCCACCGGATGGGTAAACAGTCTTG
 GCGTTTCGCTAAATACTGGCAGCGGTTTGGTCAGTATCCCGGTTTACAG/GCGGCTTGGTCTAATAATG
 GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAACCGCAACCGTGGTCGGCTTACGGCGGTGATTT
 TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGSCACCGCGCATCCAG
 Lac-TR2
 2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)
 BioLac-TR1
GGCTTTGGCTACCTGGAGAGACGGCCCGCTGATCCTTTGGGAATACGCCACCGGATGGGTAAACAGTCTTG
 CGGTTTGGCTAAATACTGGCAGCGGTTTGGTCAGTATCCCGGTTTACAG/GCGCTGCTGCTGTTGCTGCTGCT
 Splice junction
 HCGR2
GAGCATGGGCGGACATGGGCATCCAGGAGCCACTTGGGCCACGGTGCGG

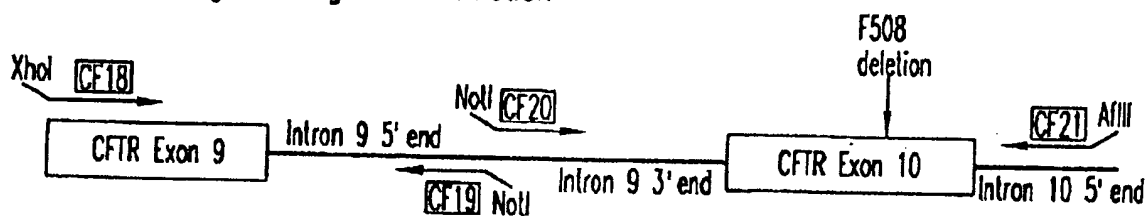
FIG.12B

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CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

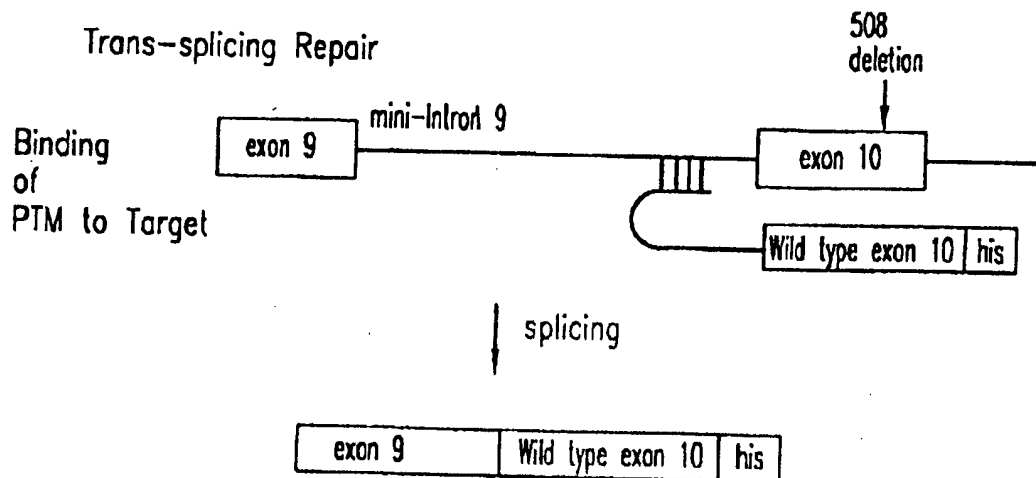


FIG.13

204070" 25474660

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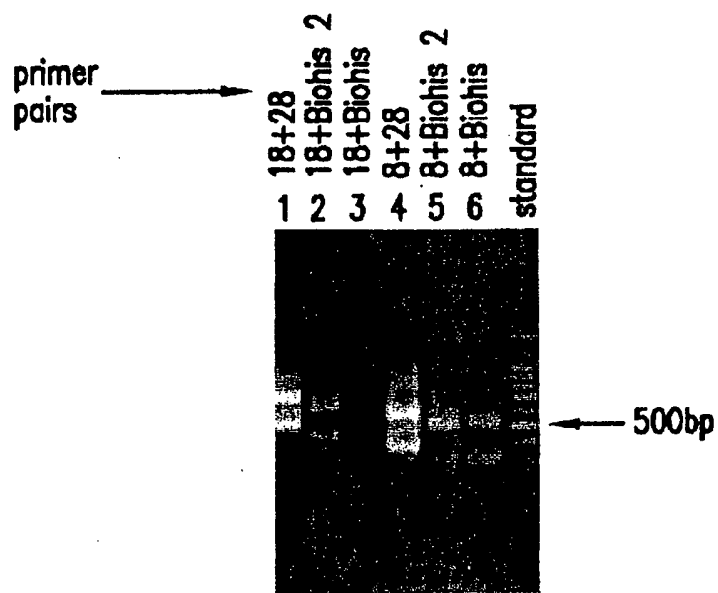


FIG.14

Positions of Restriction Endonucleases sites (unique sites underlined)

Kpn I
 Pst I
 3'ss
 102
 82
 EXON 10 CFTR + HIS TAG + STOP
 160
 240
 320
 F508
 Dde I
 Xmn I
 Sph I
 172
 190
 282
 HIS
 STOP

FIG. 15A

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Sac I
Ban II

Hae III
Not I
Pst I
EcoR V
EcoR I
Sau3A I
Dpn I
BamH I
Kpn I
Hind III
Dra I

CGGGCCGCTGCTGGATATTCGCAGAAATCCACCACACTGCACTAGTGGATCCGAGCTCGGTACCAAGCTTAAGTT 400
CGCGCGGTGACACGACCTATAGACGCTCTTAAGGTGGTGACCTGATCACCTAGGCTCGAGCCCATGGTTCGAATTCAA

321 323 339 344 349 372 373 378 379 384 390 399

CF28

Sau3A I
Dpn I

TAAACGGCTGATCAGCCTCGACTGCGCTTCTAGTTGCCAGCCAICGTGTGCCCCCTCCCGTCCCTTCCCTTGACC 480
ATTTGCCGACTAGTCCGAGCTGACACCGGAAGATCAACGGTCCGTAGACAACAACGGGAGGGGGCAGCGAAGCACTGG

410 410

CF27

CGGACGGTGGCAGCTCCCAC 500
GACCTTCCACGGTGAGGGTG

PRESENT IN PTM 3'UT
BUT NOT TARGET

Restriction Endonucleases site usage

Enzyme	Site Usage
Acc I	-
Apa I	1
ApaL I	-
Avr II	-
BamH I	1
Bon II	2
Bbe I	-
EcoR I	1
EcoR V	1
Hae II	-
Hae III	2
Hinc II	-
Hind III	1
Hinf I	-
Nde I	1
Nhe I	1
Not I	1
PfIM I	-
Pst I	2
Pvu I	-
Pvu II	-
Sau96 I	2
Sca I	1
Sma I	1
Sph I	1
Spl I	-
Ssp I	-
Stu I	-

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+

CFTR Target
(mini-gene)



F508 deletion

Mini-intron 9
(~0.6kb)

Cotransfect PTM and target molecules in HEK 293 cells
and detect repaired CFTR mRNA by RT-PCR.

Repaired
CFTR mRNA

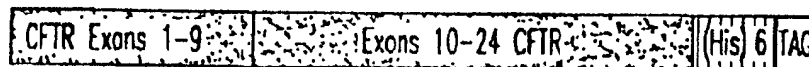


FIG.16

204040" 26474660

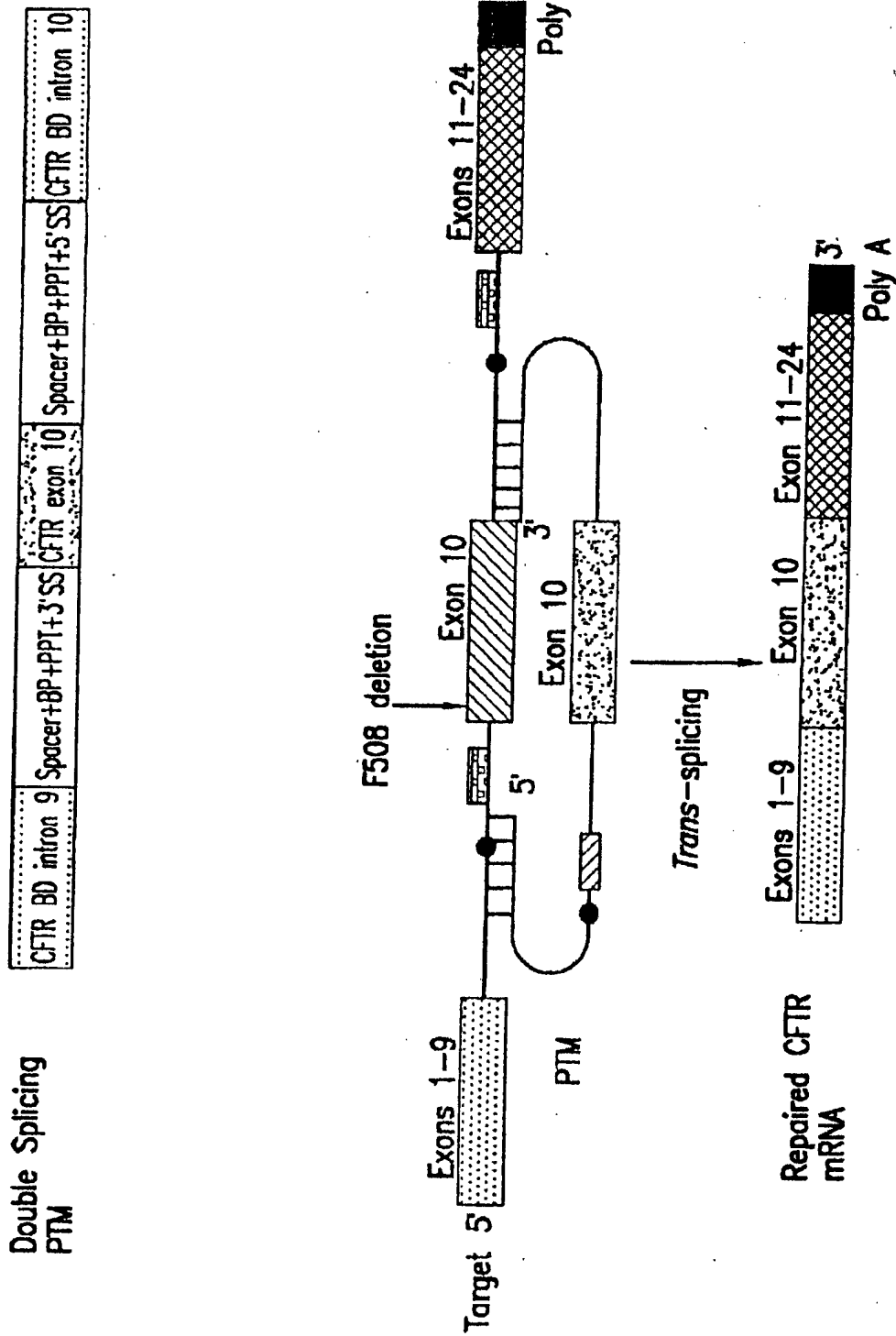


FIG.17

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DOUBLE TRANS-SPLICING SPECIFIC TARGET

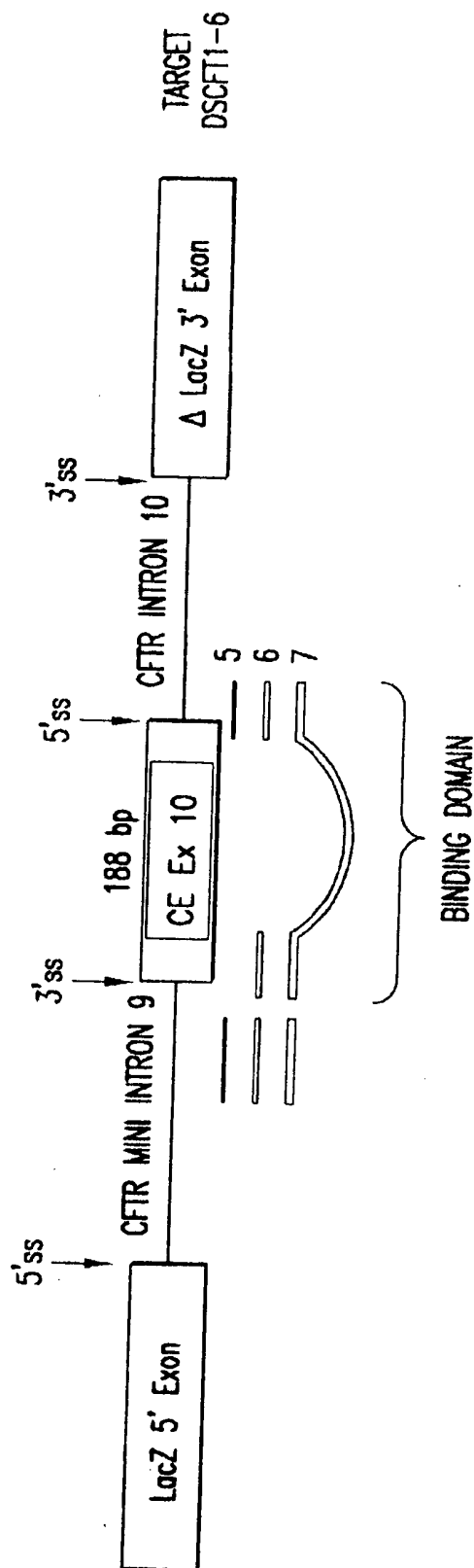


FIG.18

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DOUBLE TRANS-SPLICING PTMS

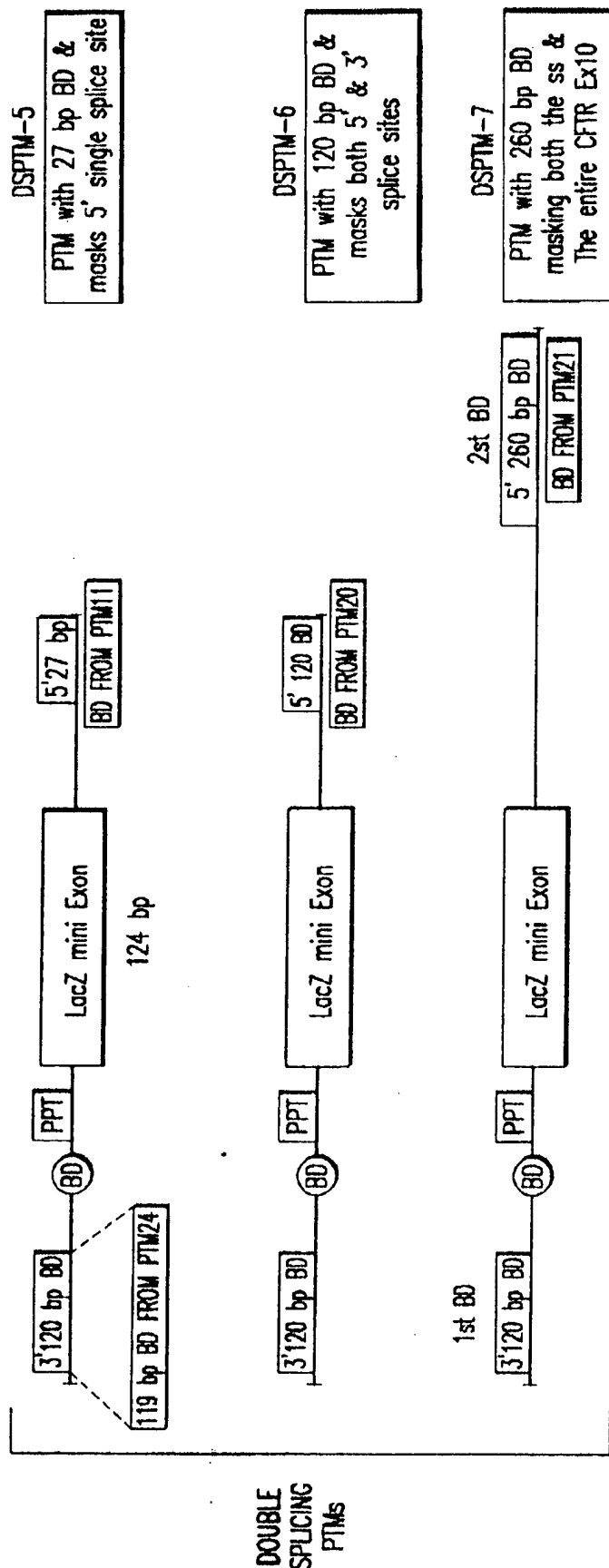
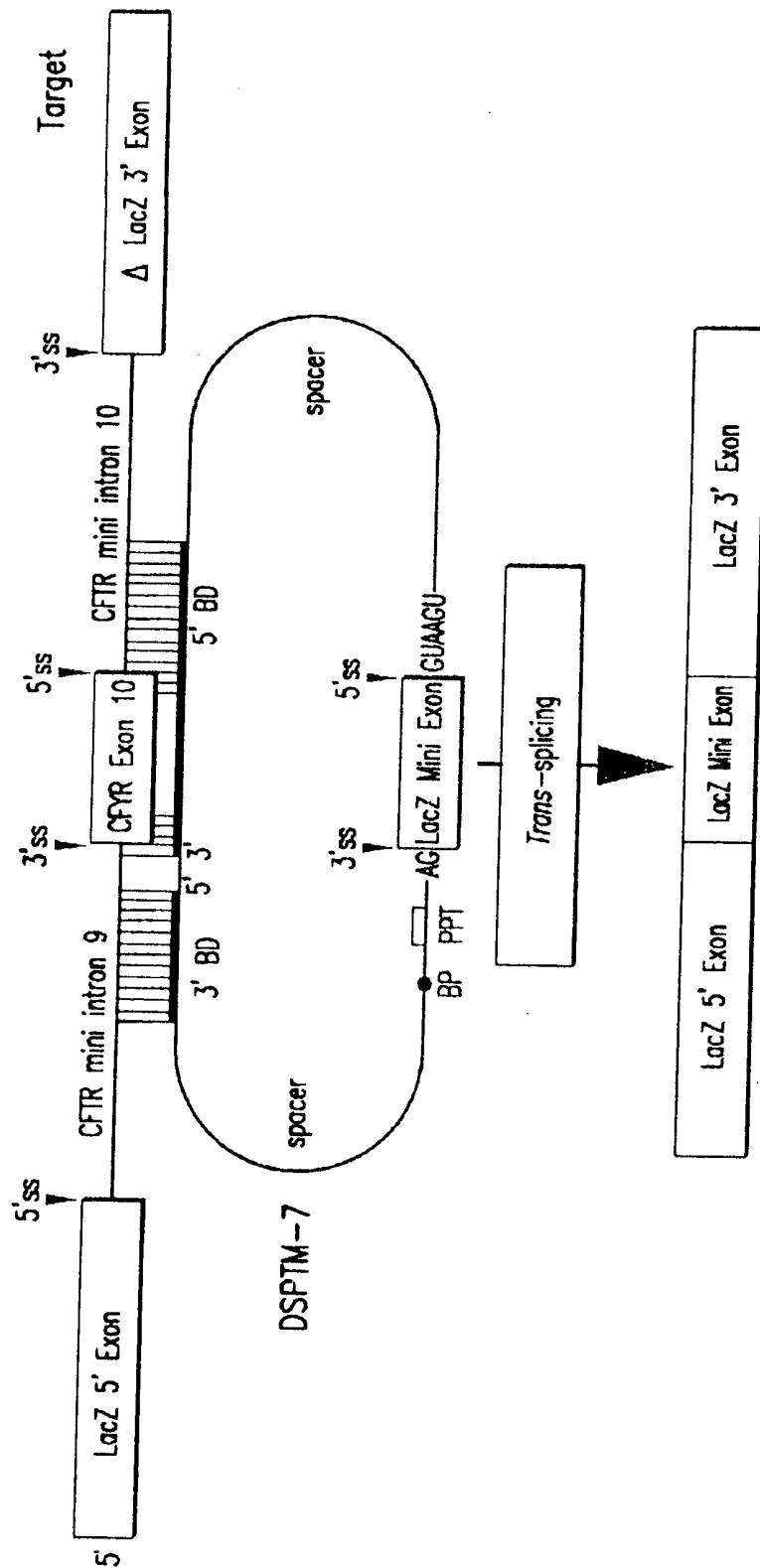


FIG.19

204040" 2577150

DOUBLE TRANS-SPLICING β -GAL MODEL



Repaired LacZ mRNA

FIG.20

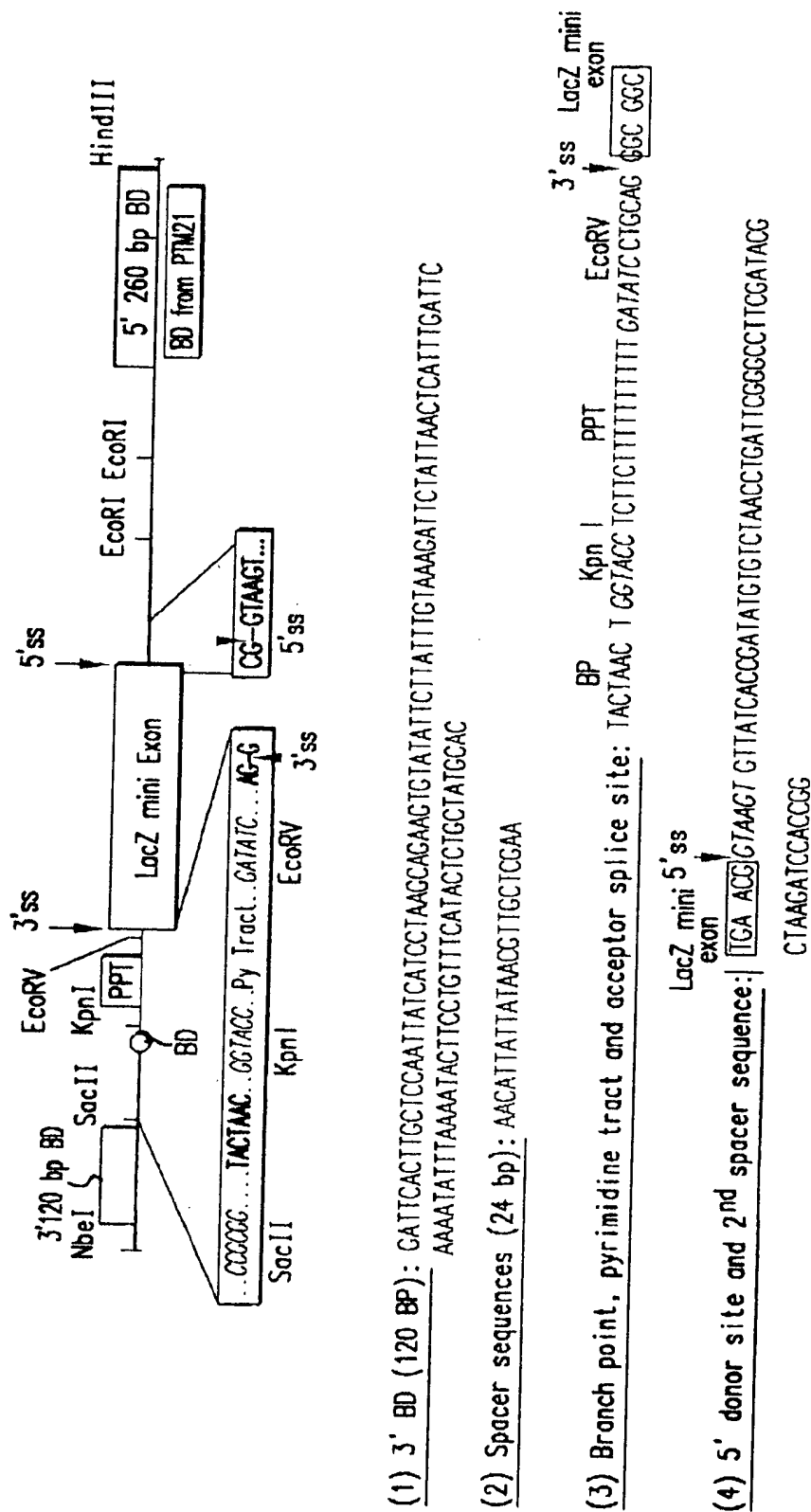
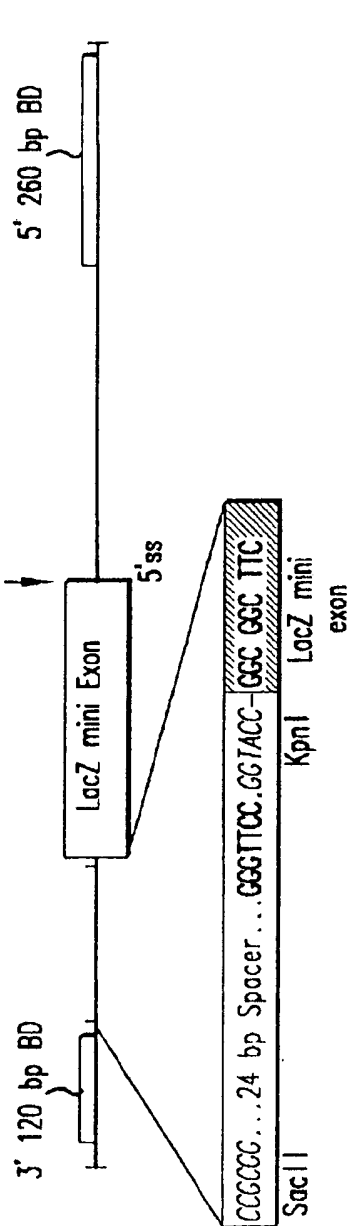
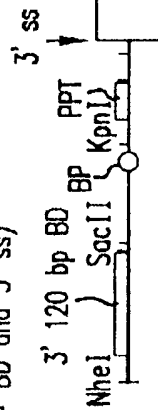


FIG.21

DSPTM8: (Δ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



FIG.22

Mutants

ACCURACY OF DOUBLE TRANS-SPICING REACTION

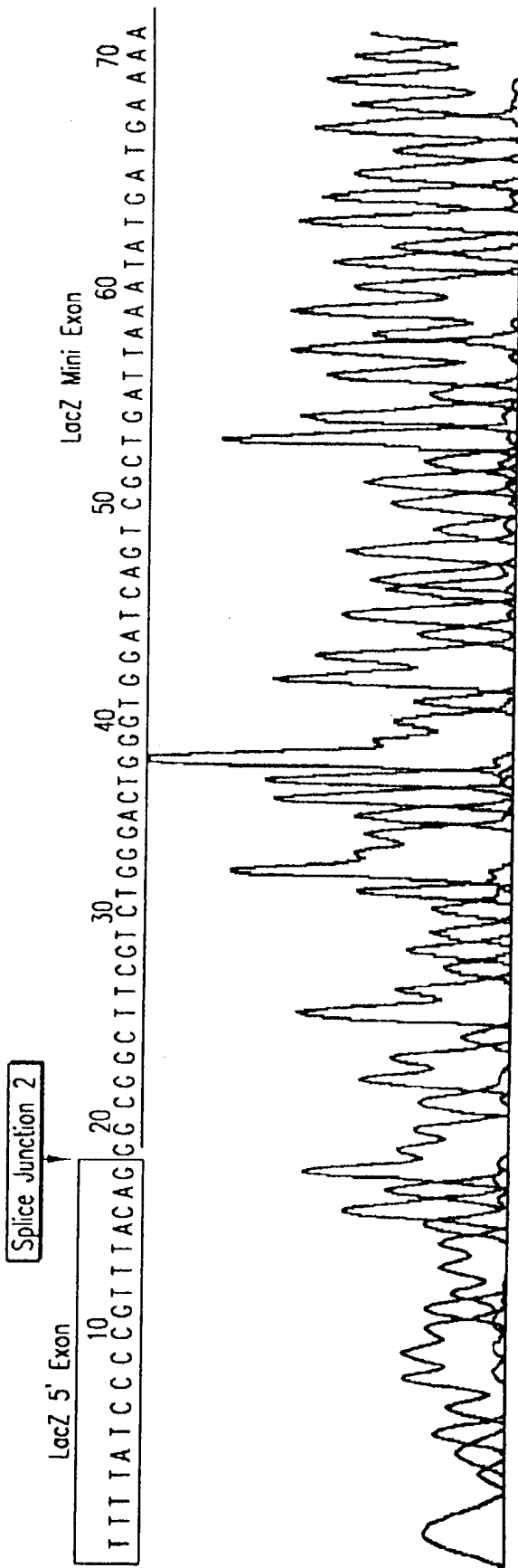


FIG.23A

ACCURACY OF DOUBLE TRANS-SPlicing REACTION

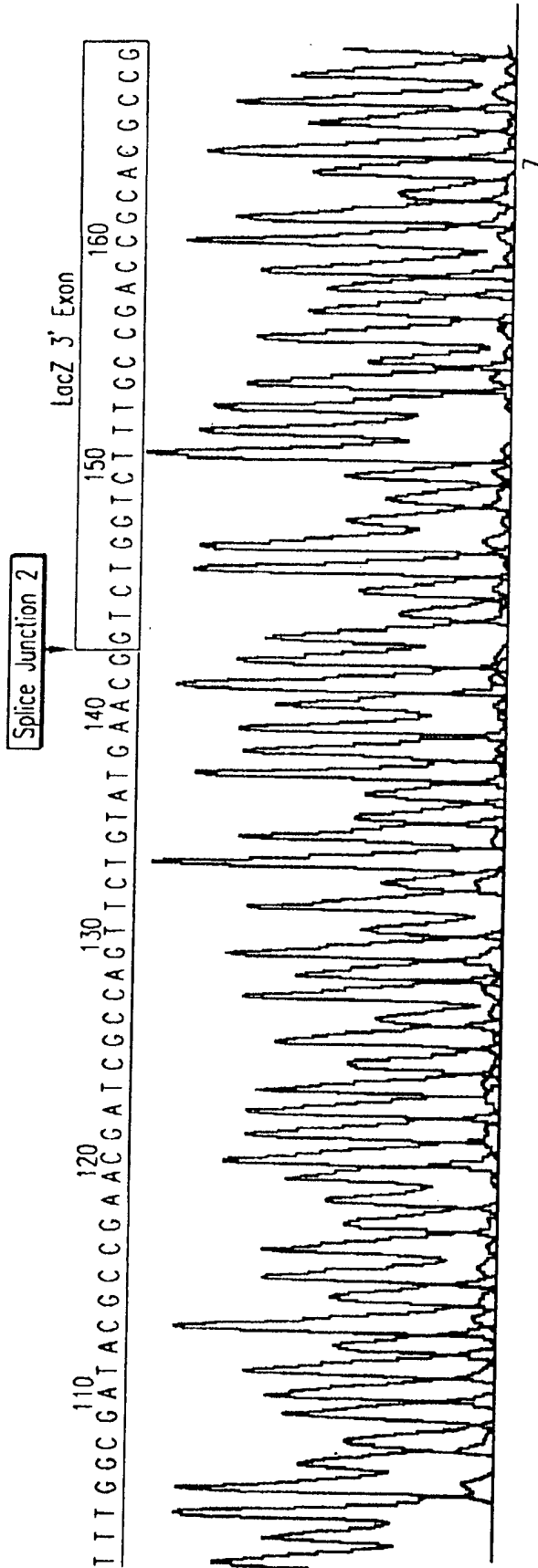
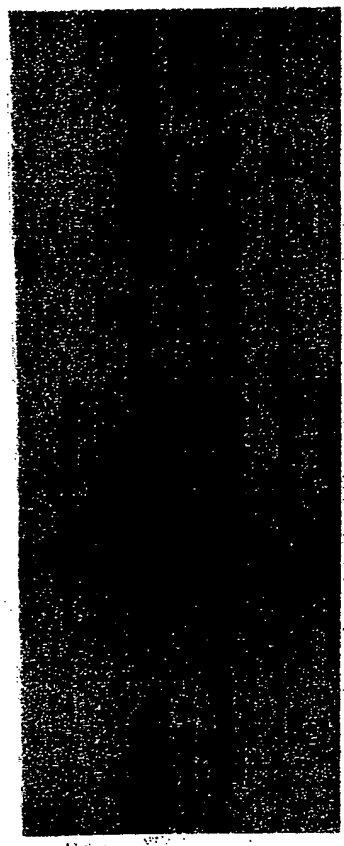


FIG.23B

Double Trans-splicing Produces Full-length Protein



β-gal →
(120 kDa)

1 2 3 4 5 6 7

Lane 1: DSCFT1.6 Target alone 25 μg
Lane 2: DSPTM7 25 μg
Lane 3 Target + PTM #6 25 μg
Lane 4: Target + PTM #9 25 μg
Lane 5: Delta 3' splice mutant alone 25 μg
Lane 6: Target + Delta 3' ss 25 μg
Lane 7: Target+PTM29+30 (mutants) 25 μg

Figure 24

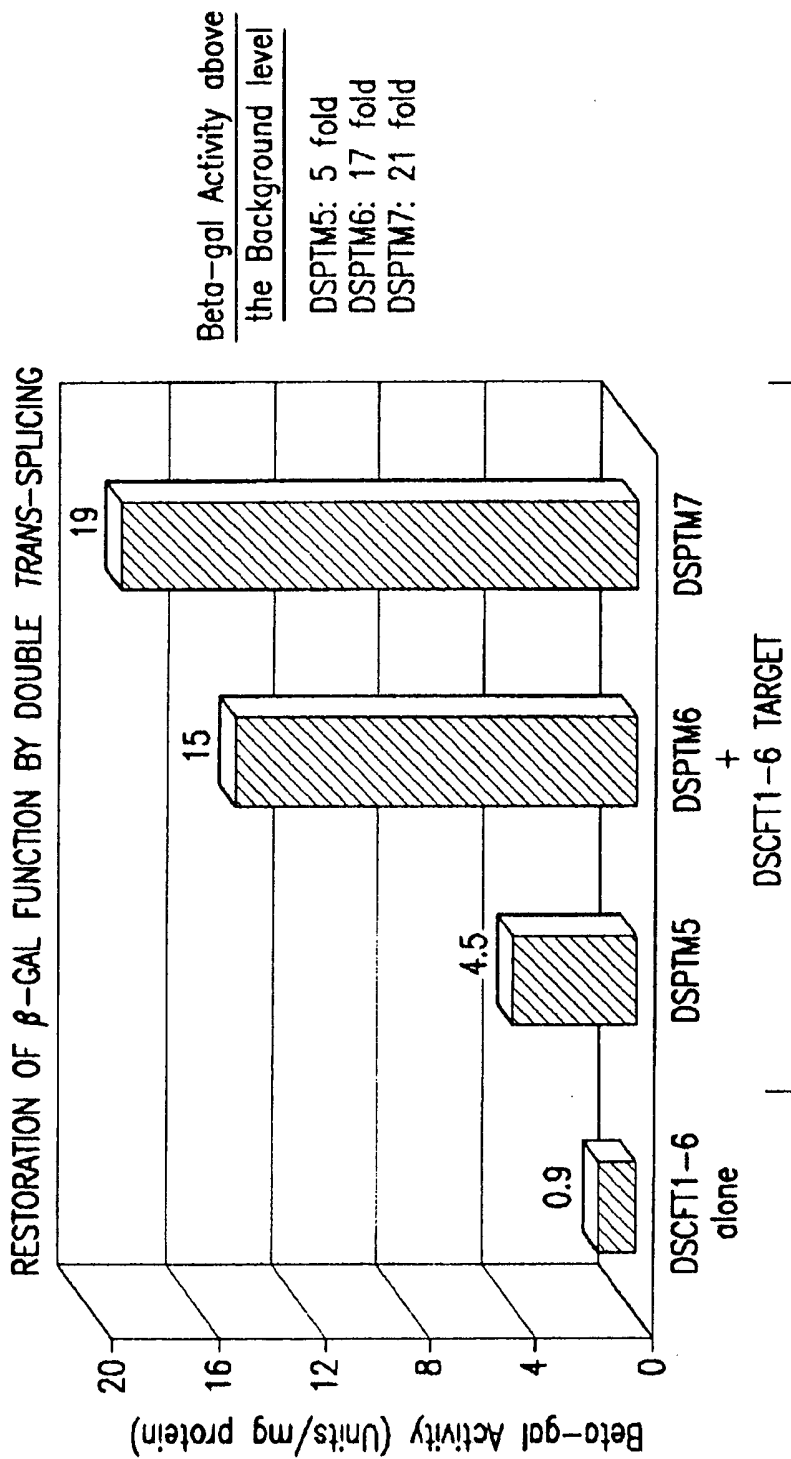


FIG.25

RESTORATION OF β -GAL ACTIVITY IS DUE TO DOUBLE RNA TRANS-SPLICING EVENTS

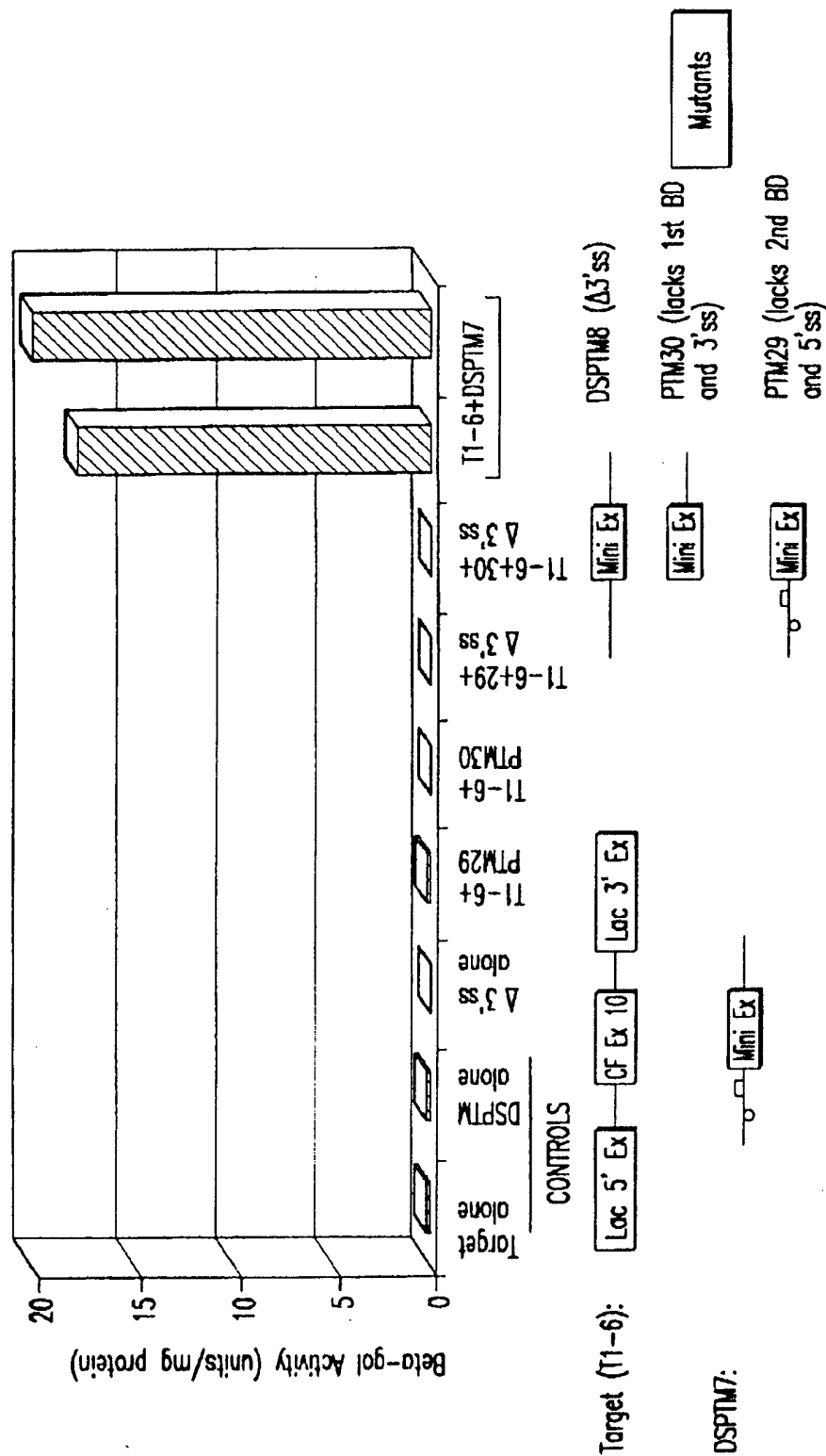


FIG.26

DOUBLE TRANS-SPLICING: TITRATION OF TARGET & PTM

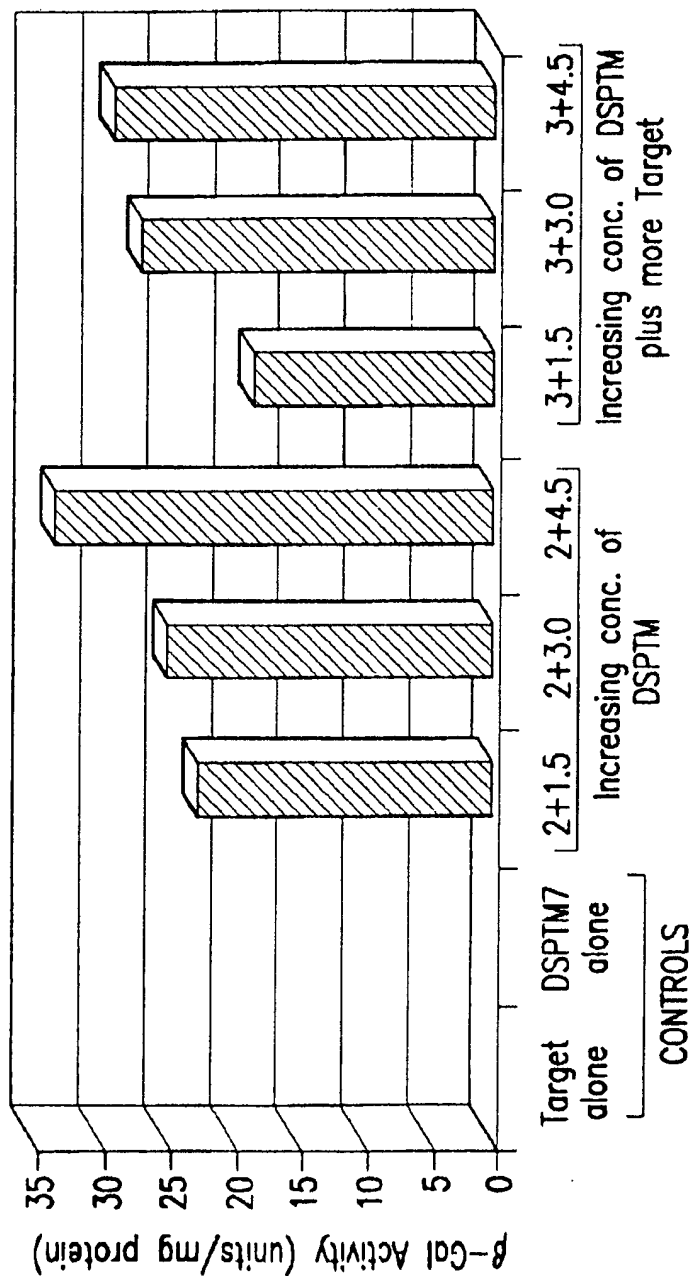


FIG.27

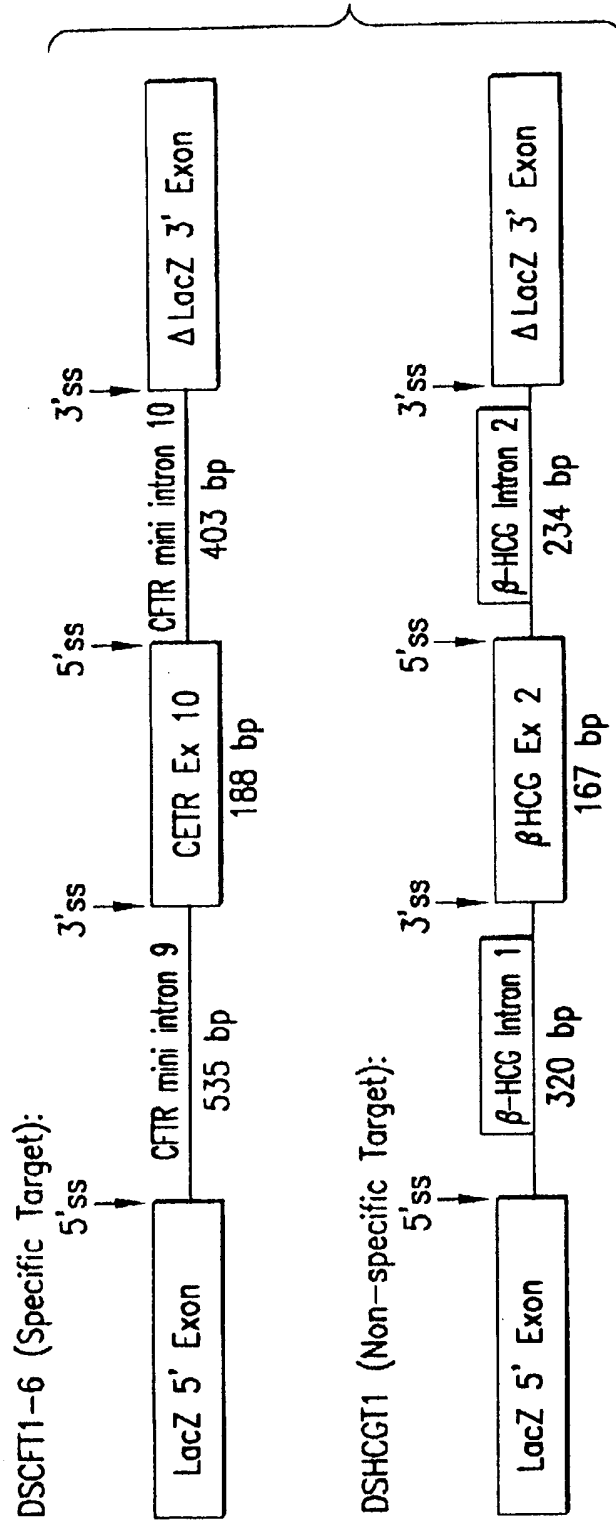


FIG.28

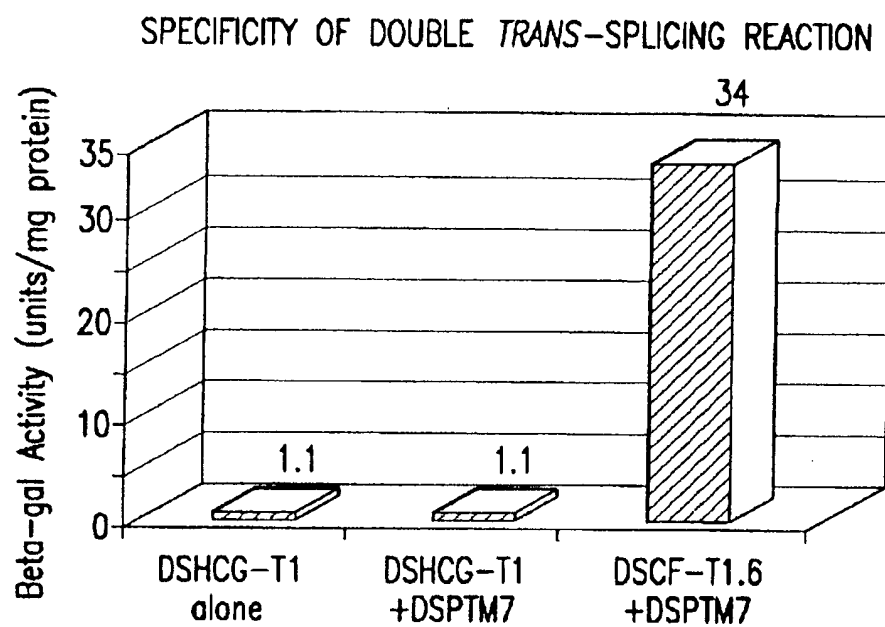


FIG.29

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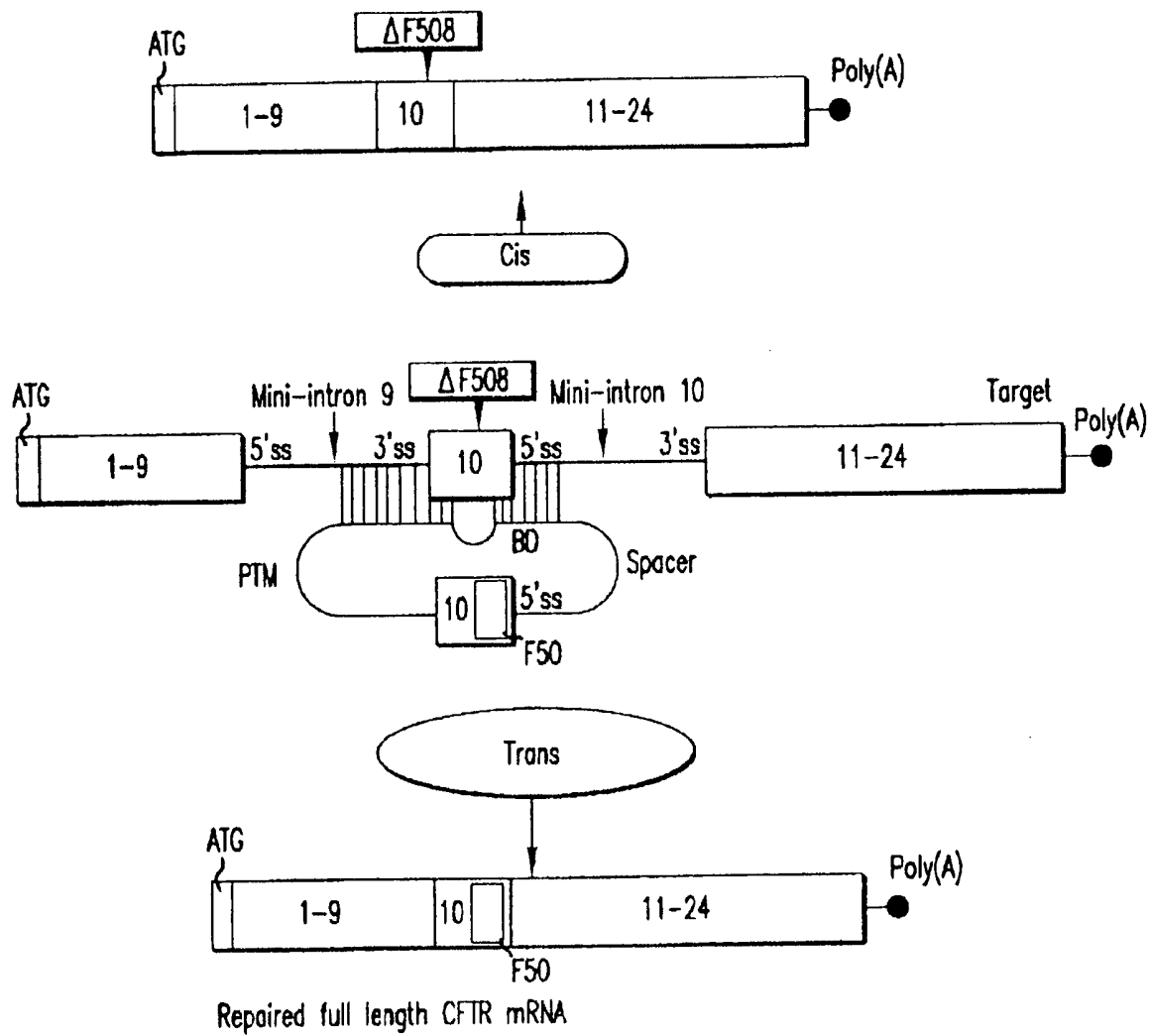
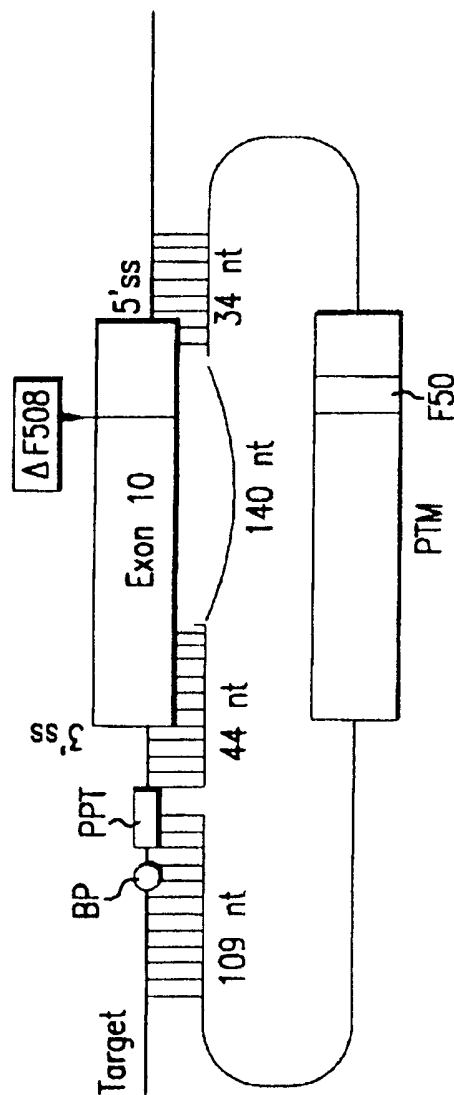


FIG.30

PTM with a long binding domain masking
two splice sites and part of exon 10
in a mini-gene target



ACGAGCTTGGTCATGATGATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAACATTCGG
GCCGATCAGCTTTTCAGCCAAATTCAGTGGATCATGCCCGGTACCATCAAGGAGAACATTAAT
CTTCGCGCTCAGTACGACGAGTACCGCTATCGCTGGTGAATTAAGGCCGTGTCAGTTCGAGGAG

MCU in exon 10 of PTM

88 OF 192 (46%) bases in PTM exon 10 are not complementary to
its binding domain (bold and underlined).

FIG.31

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Sequence of a double
Trans-spliced product

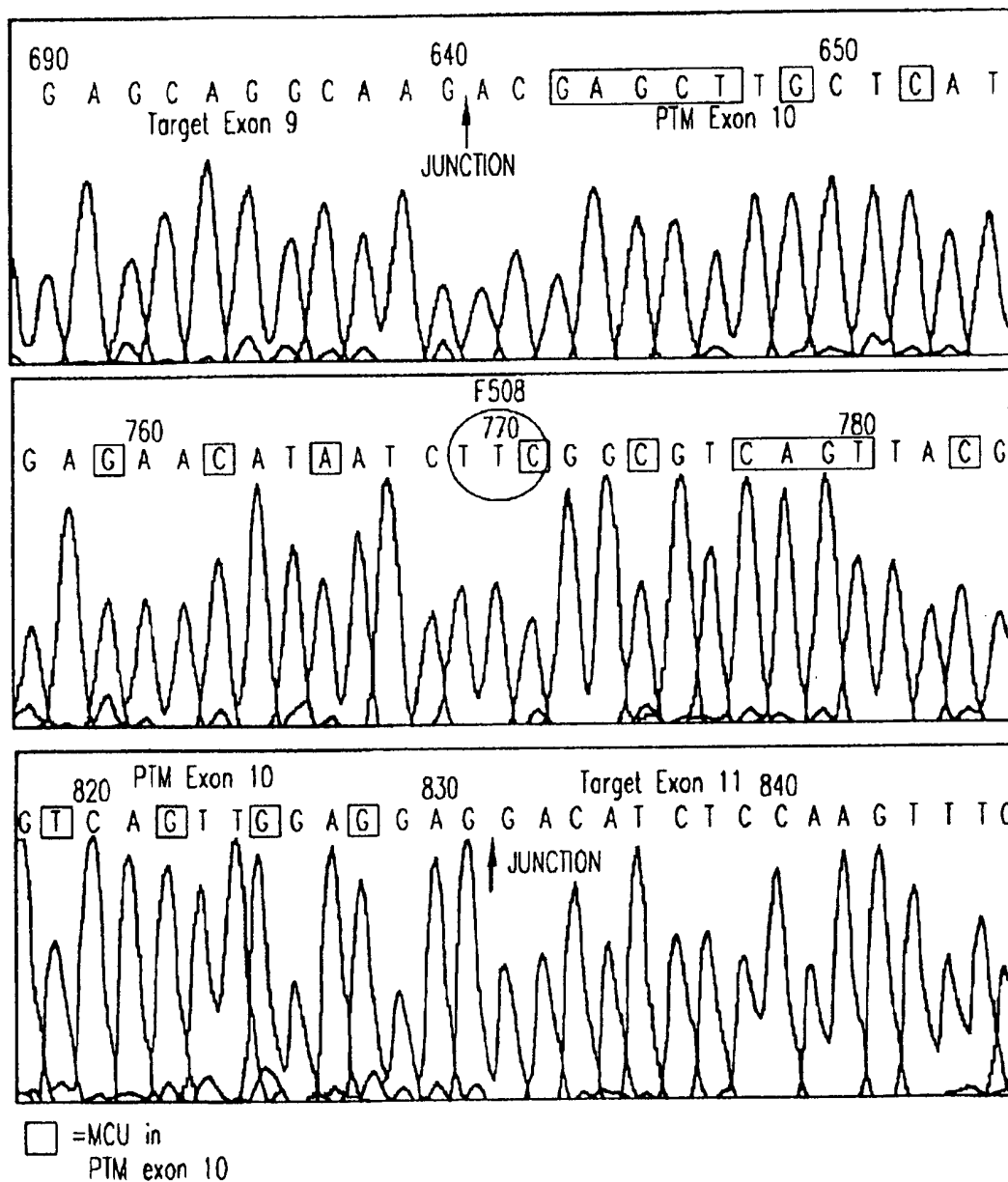


FIG.32

204040" 25474660

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

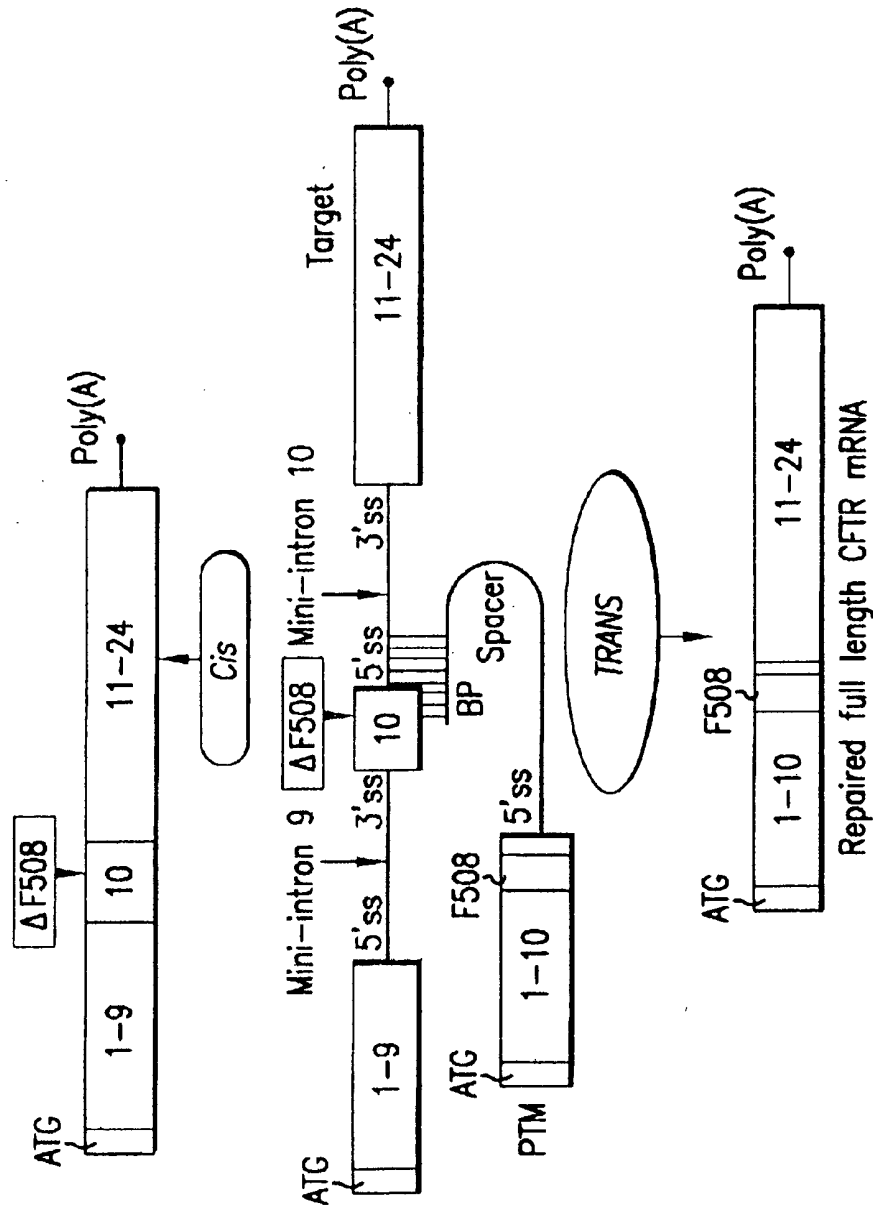


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

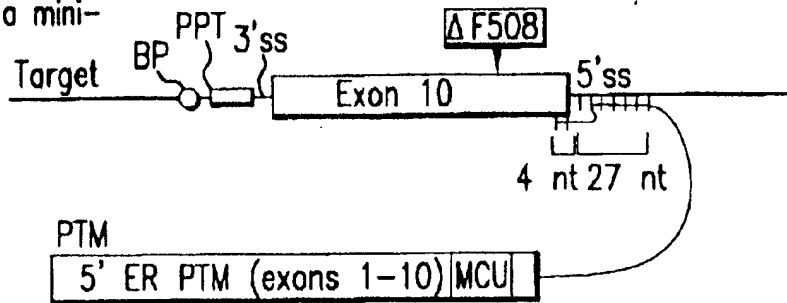


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

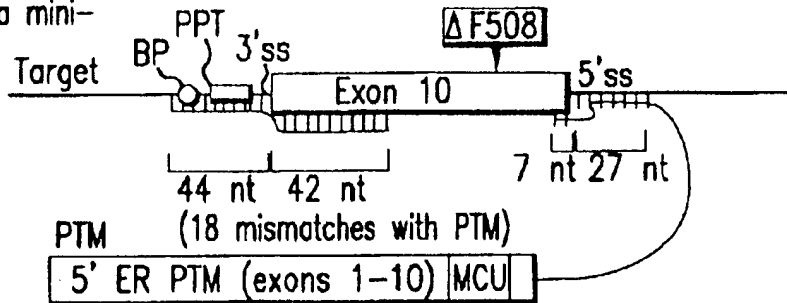


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

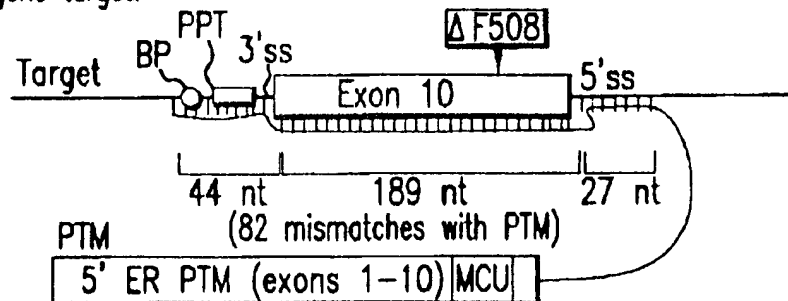
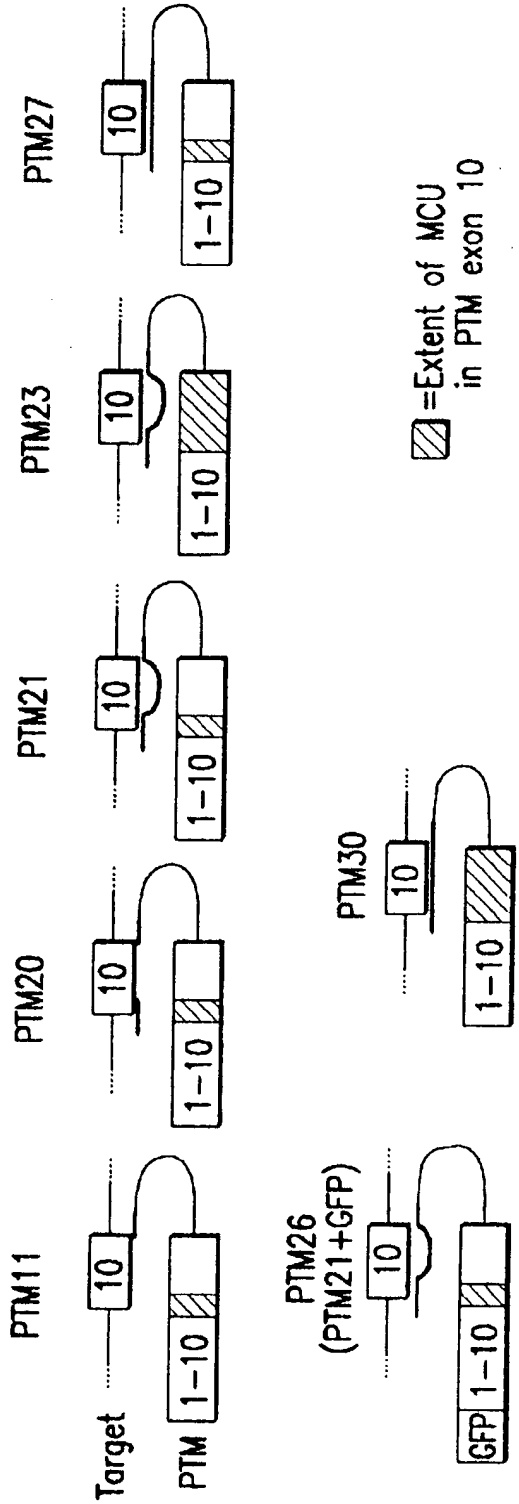


FIG.34C



MCU in exon 10 of PTM
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCATCATCATCGCGAGTTAGAACCAAGTGAAGCAAGATCAAACATTCCG
GCGCATCAGCTTTTCAGGCCAAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAAATAT
CTTCGCGGTCAGTTACGACGAGTACCGCTATCGCTGGTGTATTAAAGGCCGTGTCAGTTGGAGGAG

FIG.35

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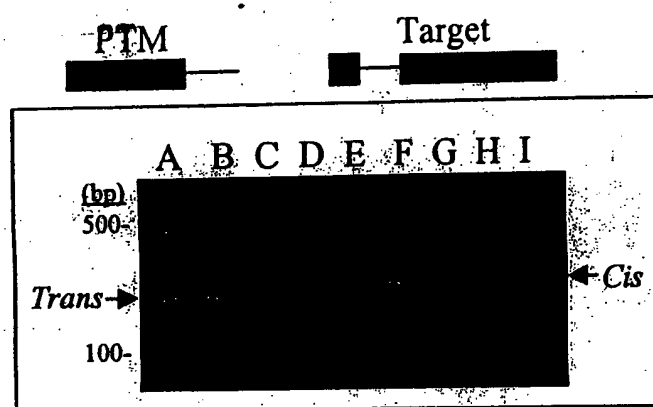


FIG. 36 A

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Cis-spliced product
[Primers CF1+CF111]

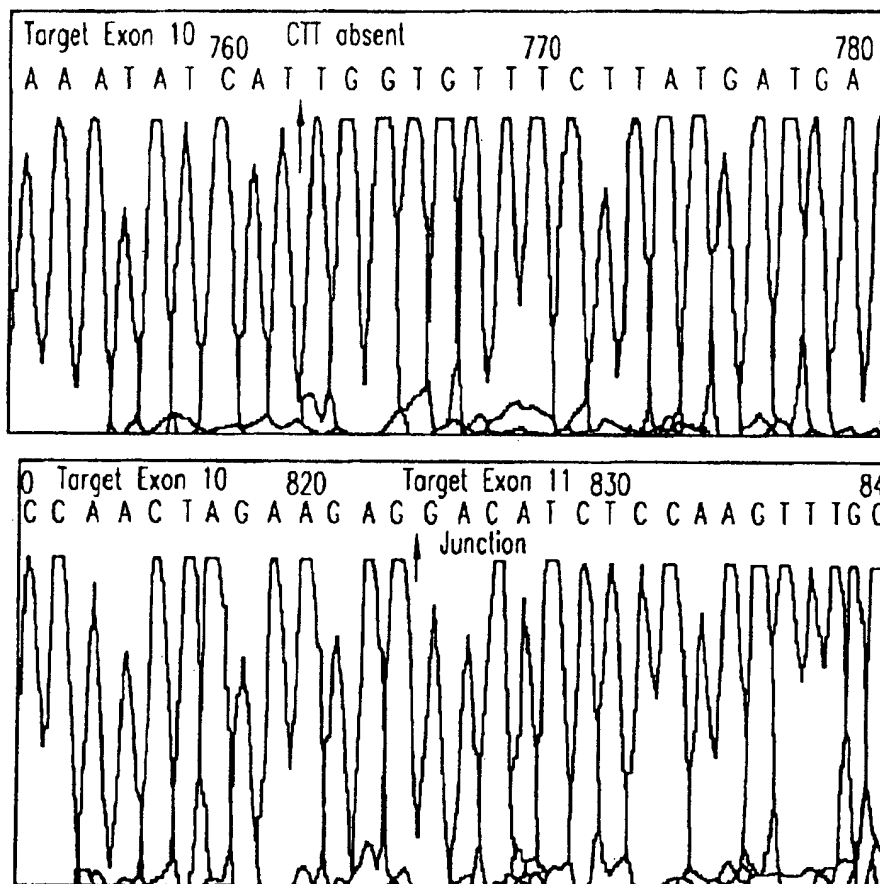


FIG.36A-1

204040" 25474660

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Trans-spliced product
[Primers CF93+CF111]

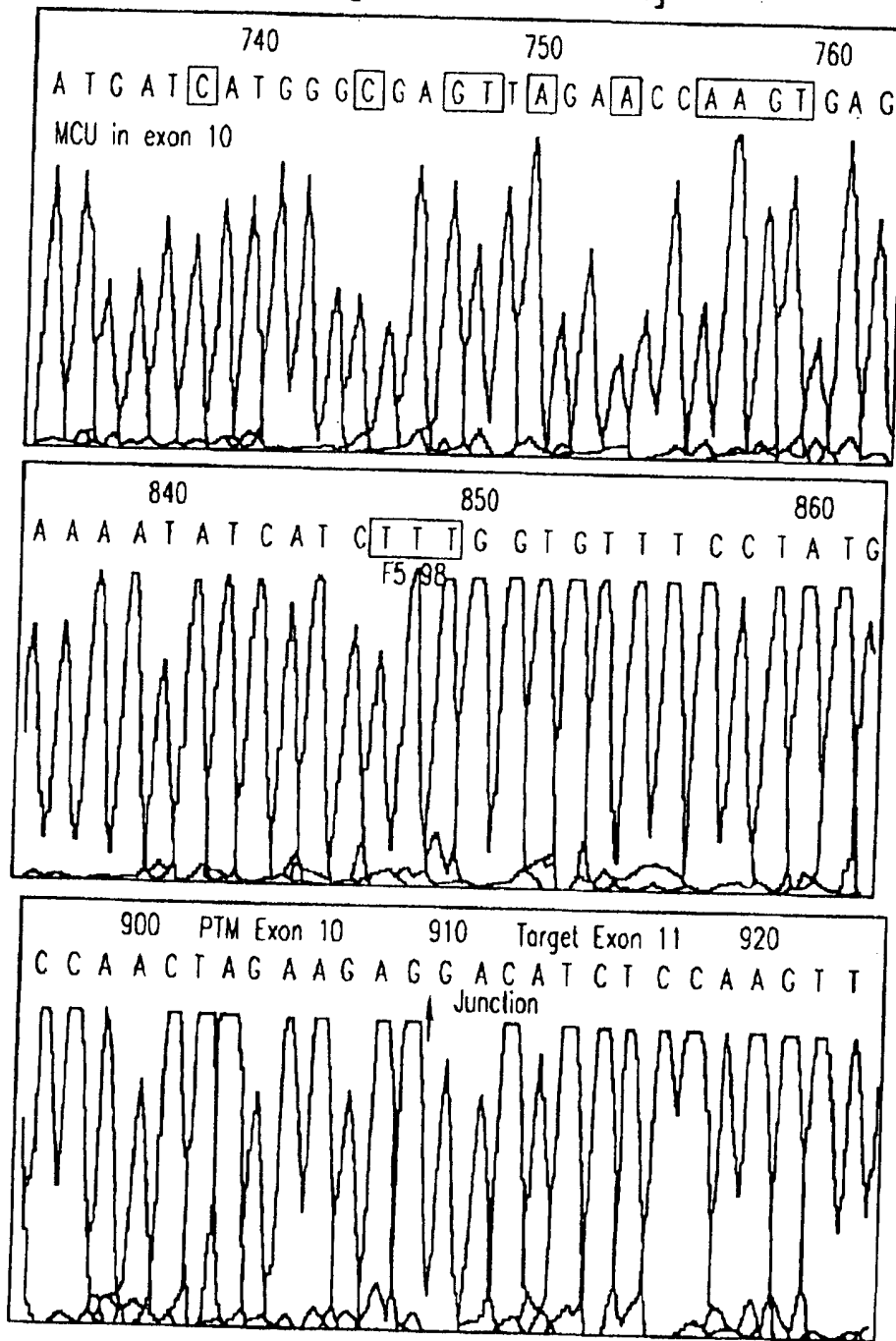


FIG.36B

204040" 25444650

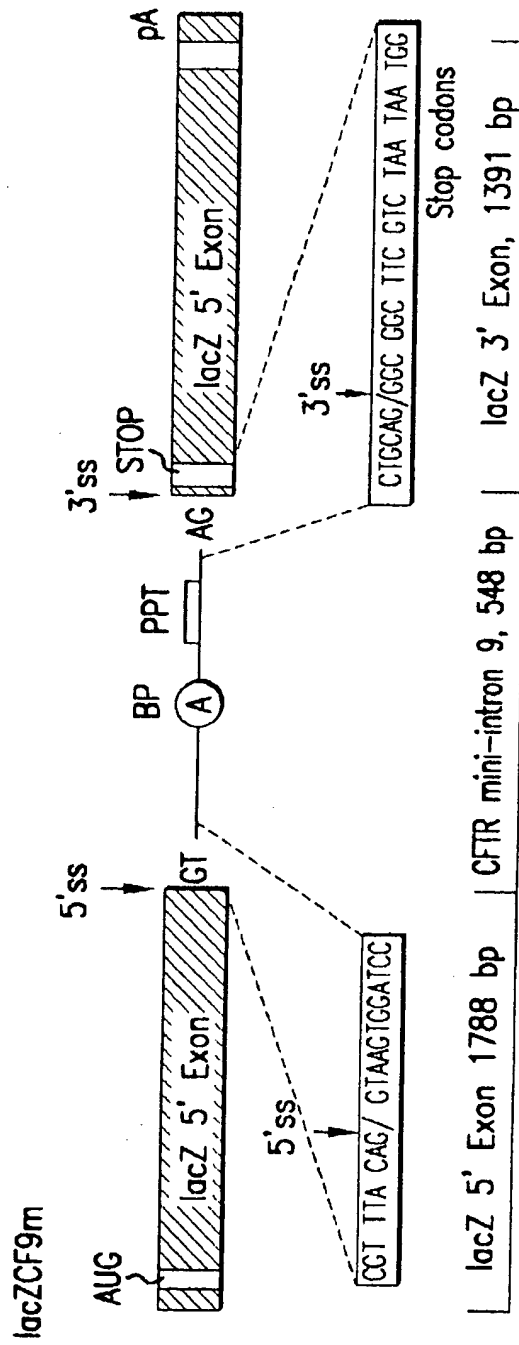


FIG.37A

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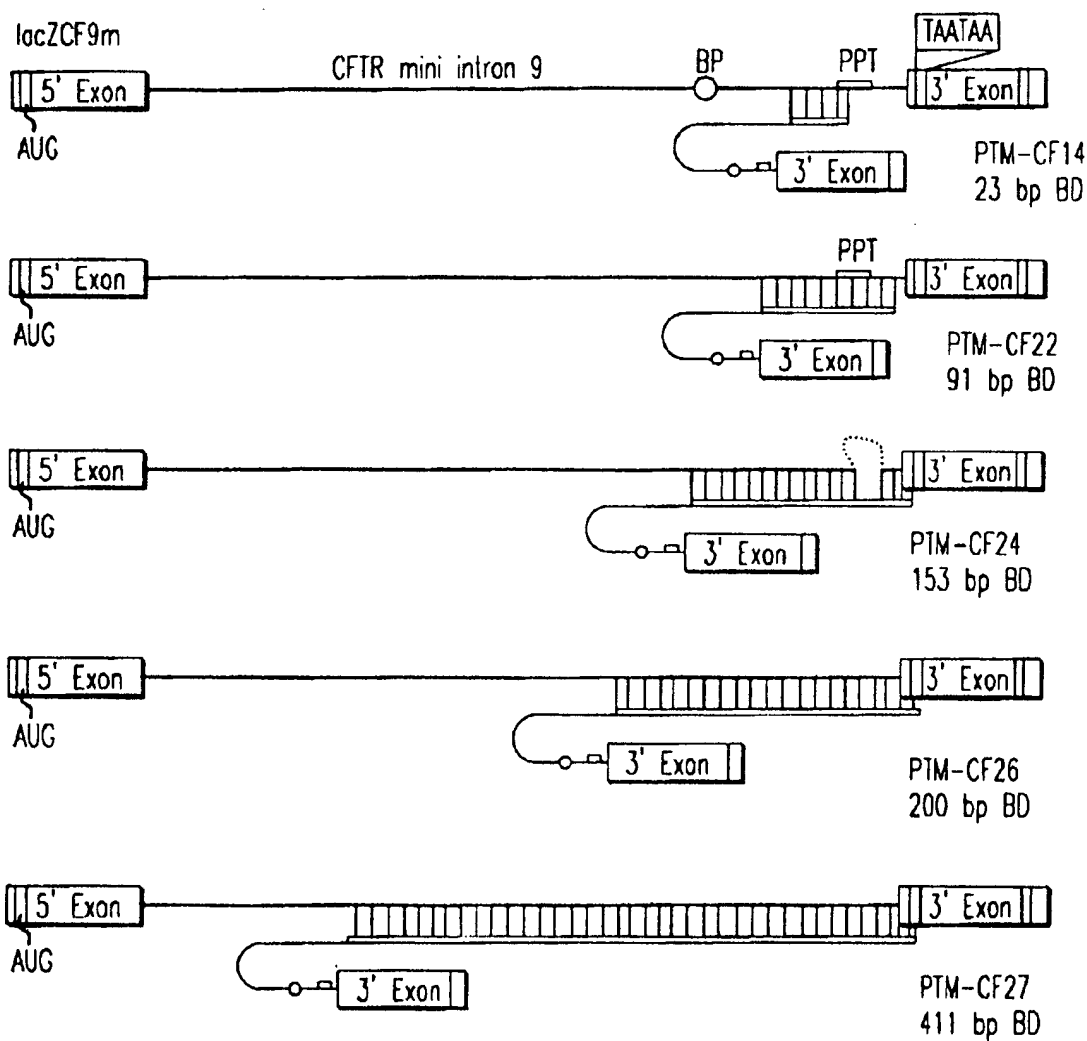
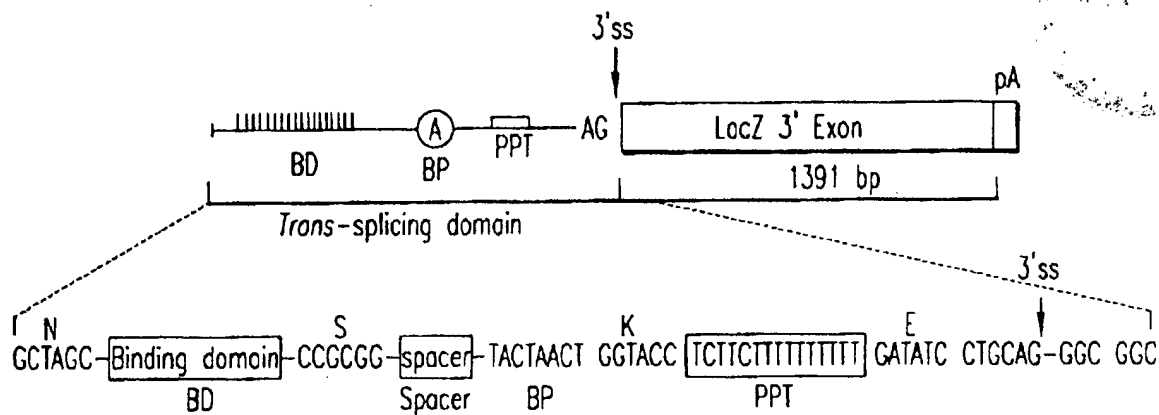


FIG.37B

202040" 267F660

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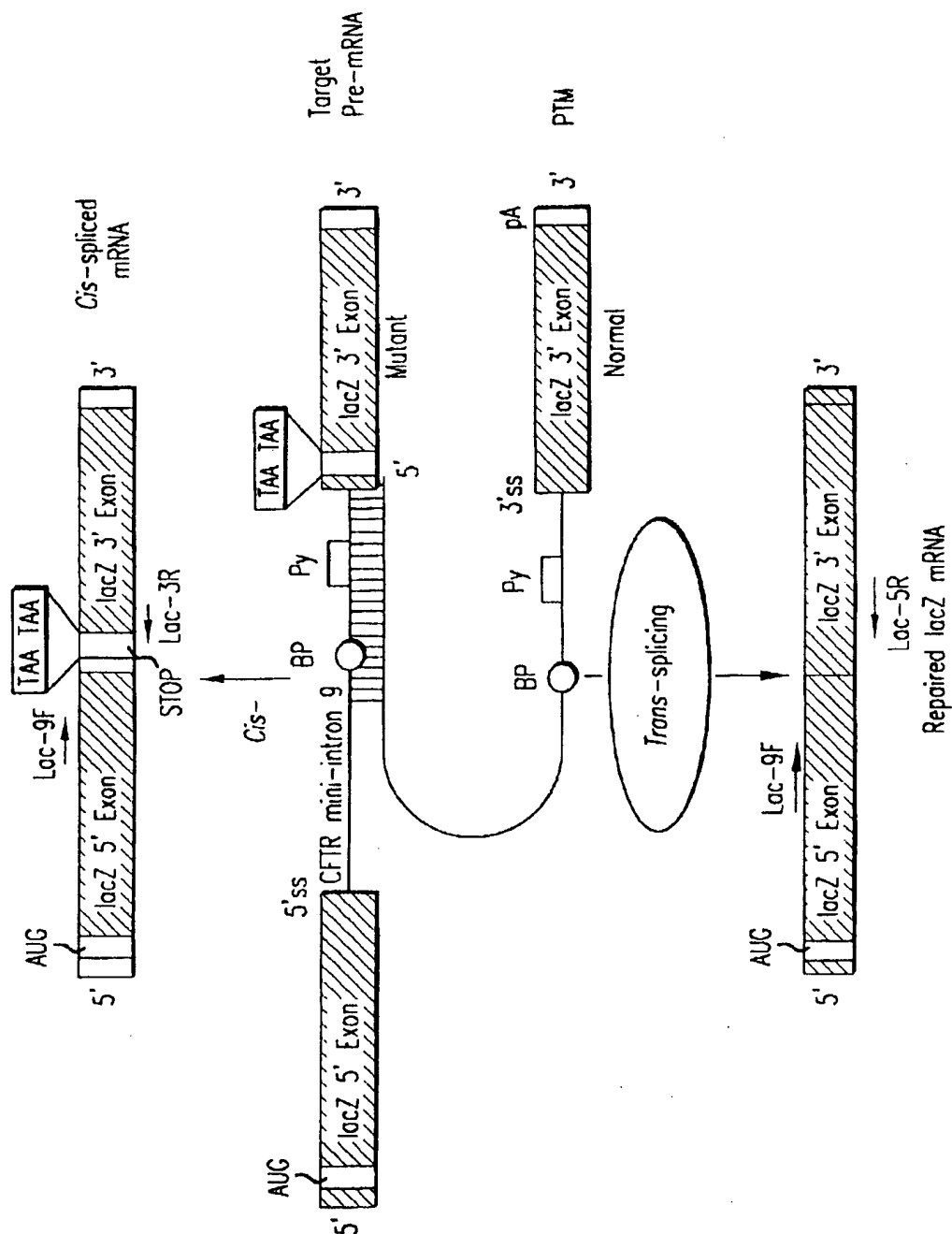


FIG.37C

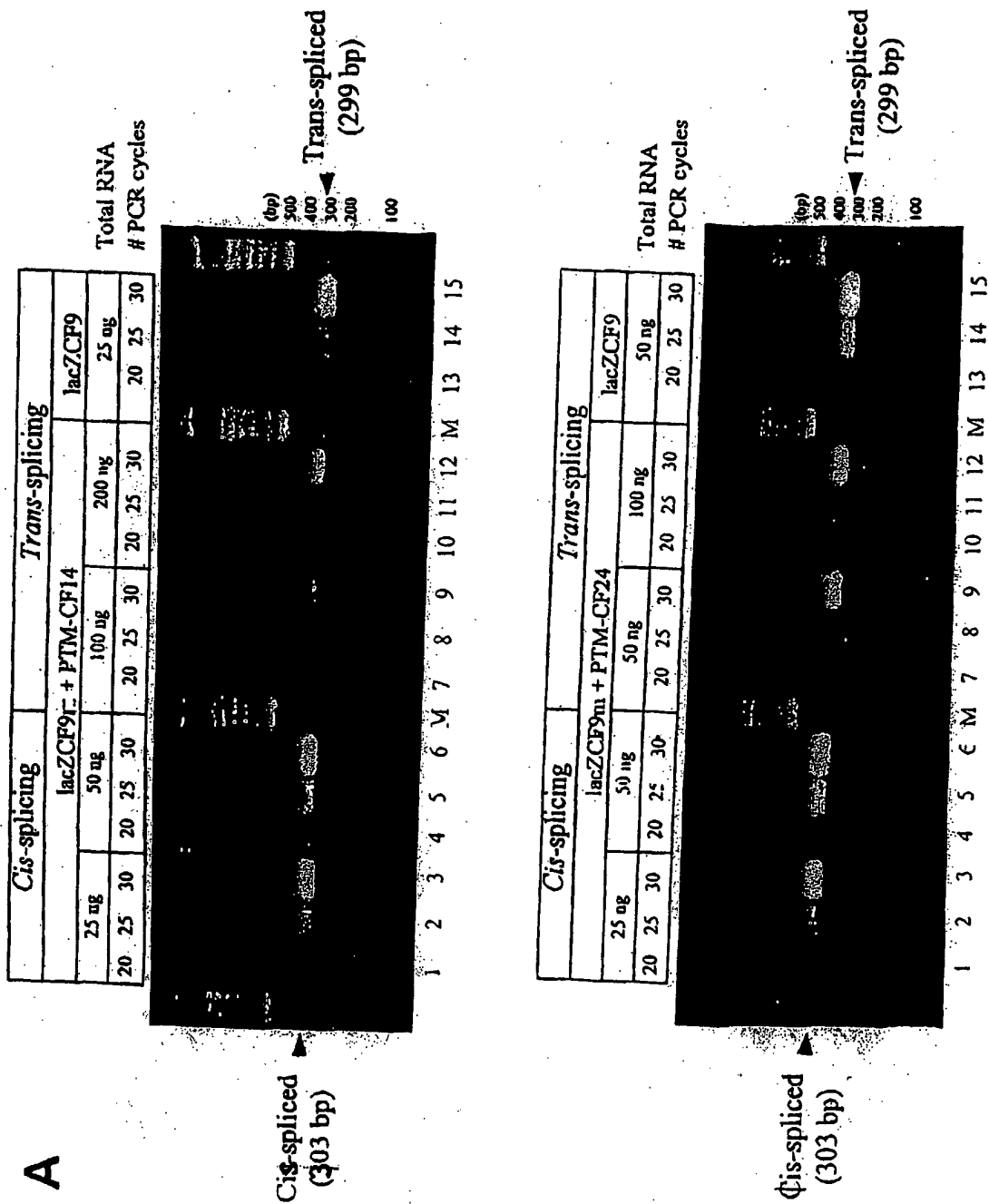


FIG. 38A

B

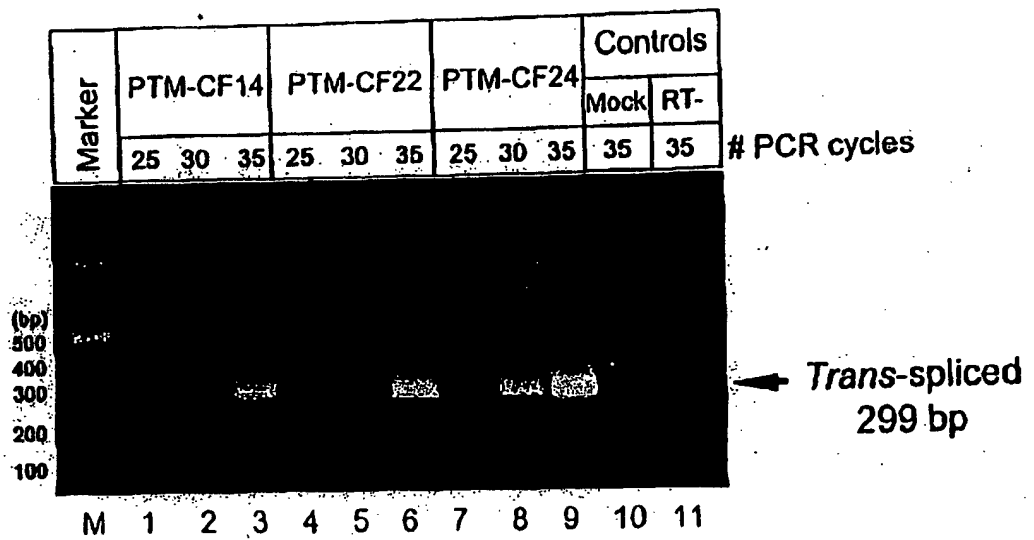


FIG. 38B

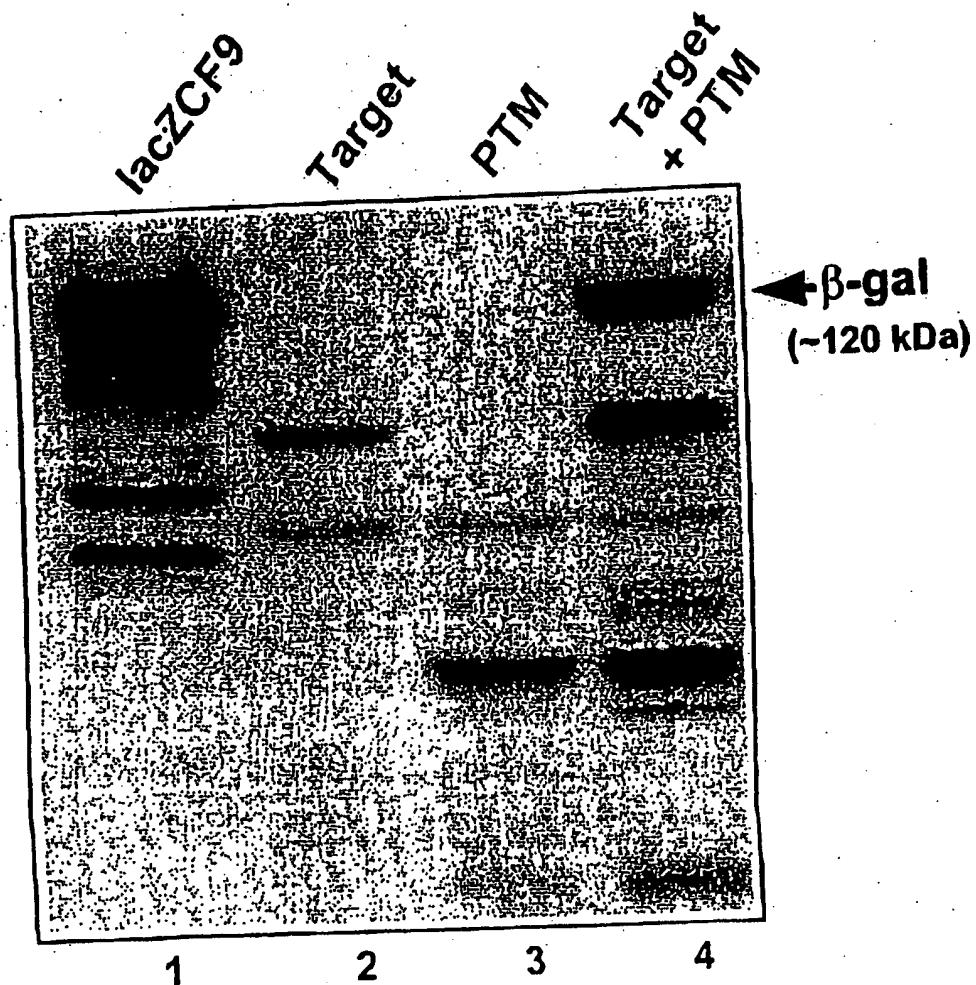


FIG. 39

204040" 254T7650

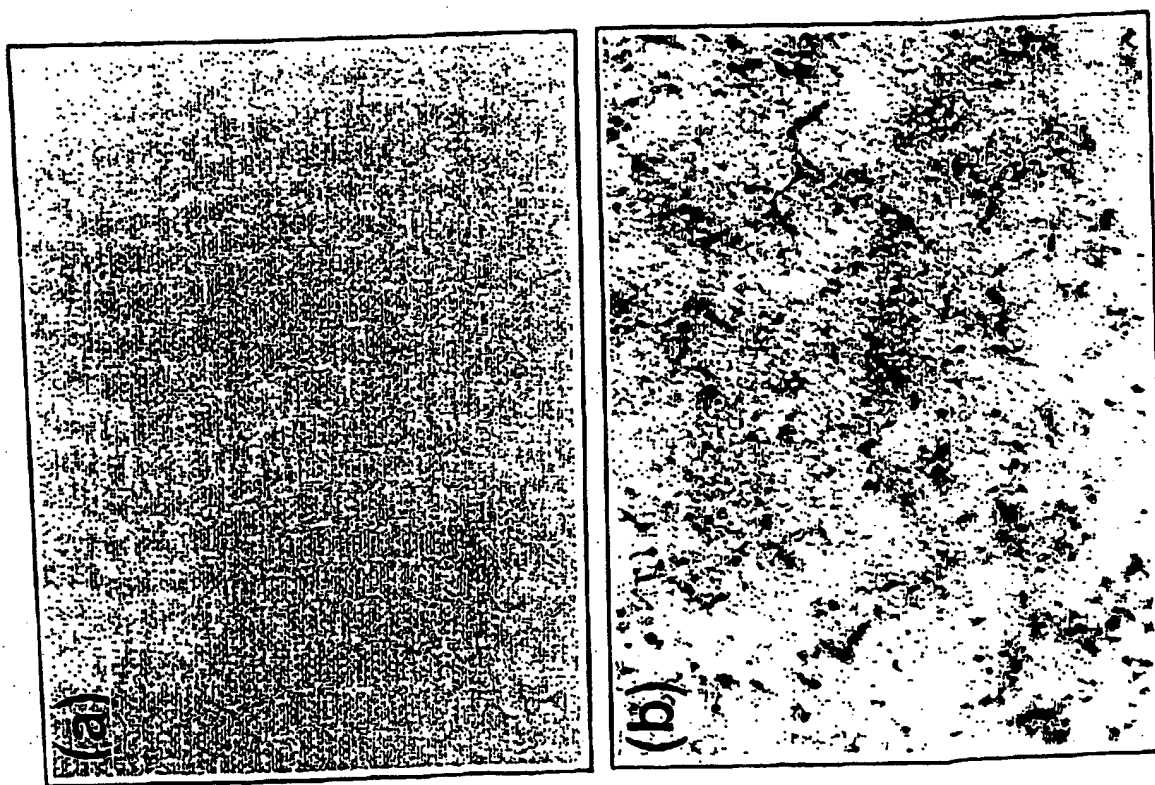


FIG. 40A

A

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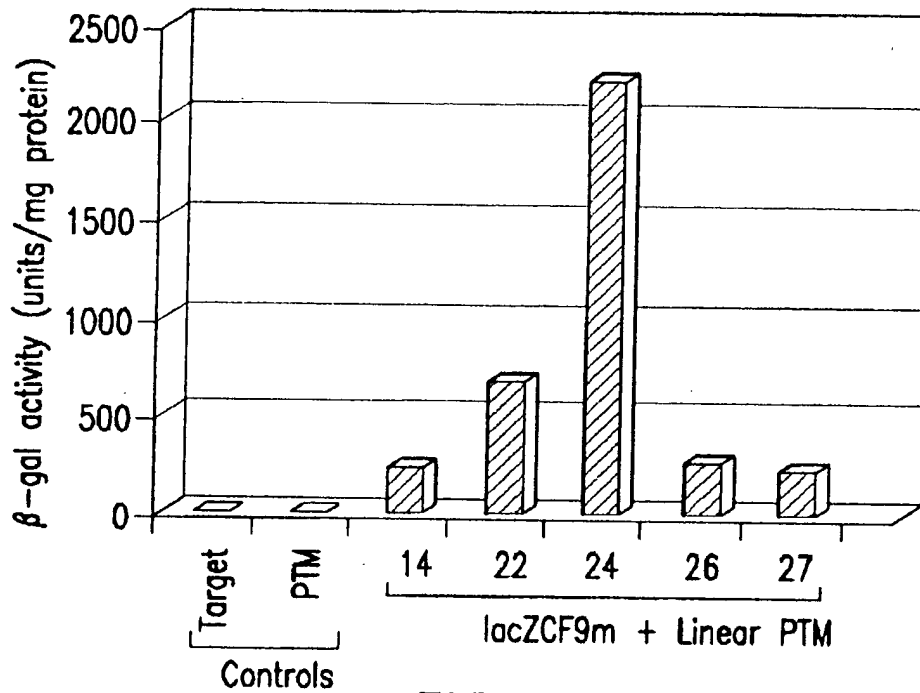


FIG.40B

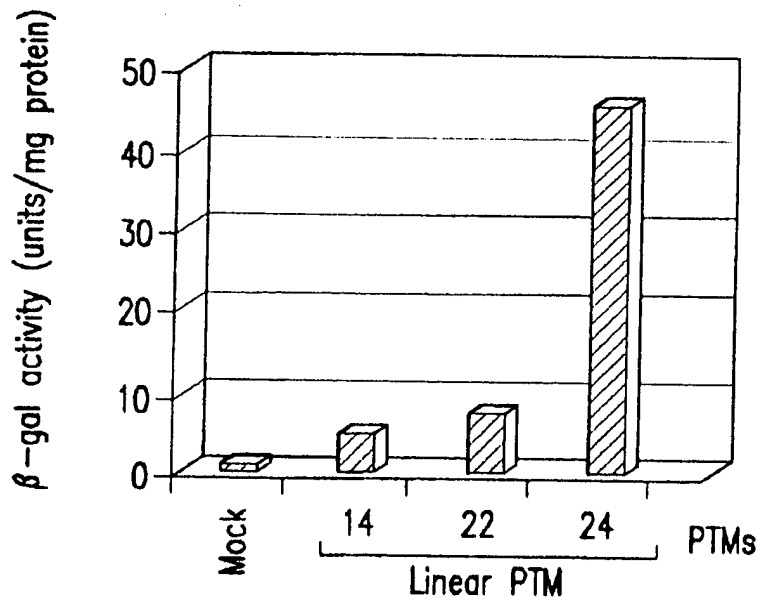


FIG.40C

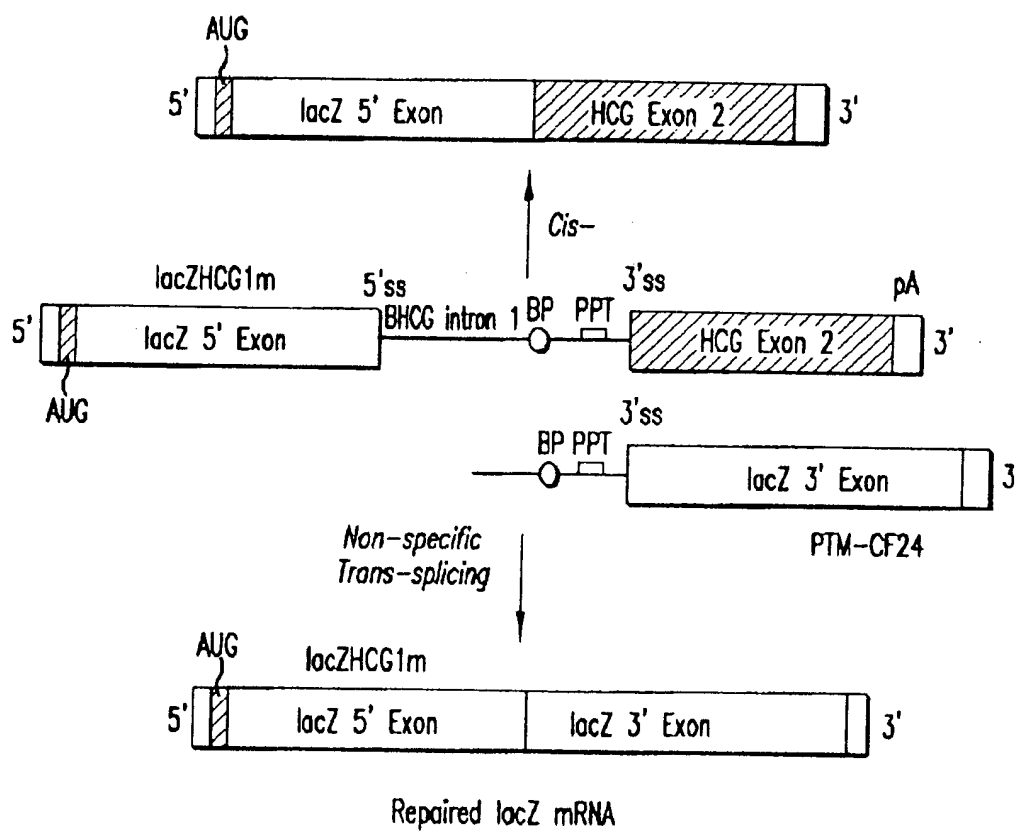


FIG.41A

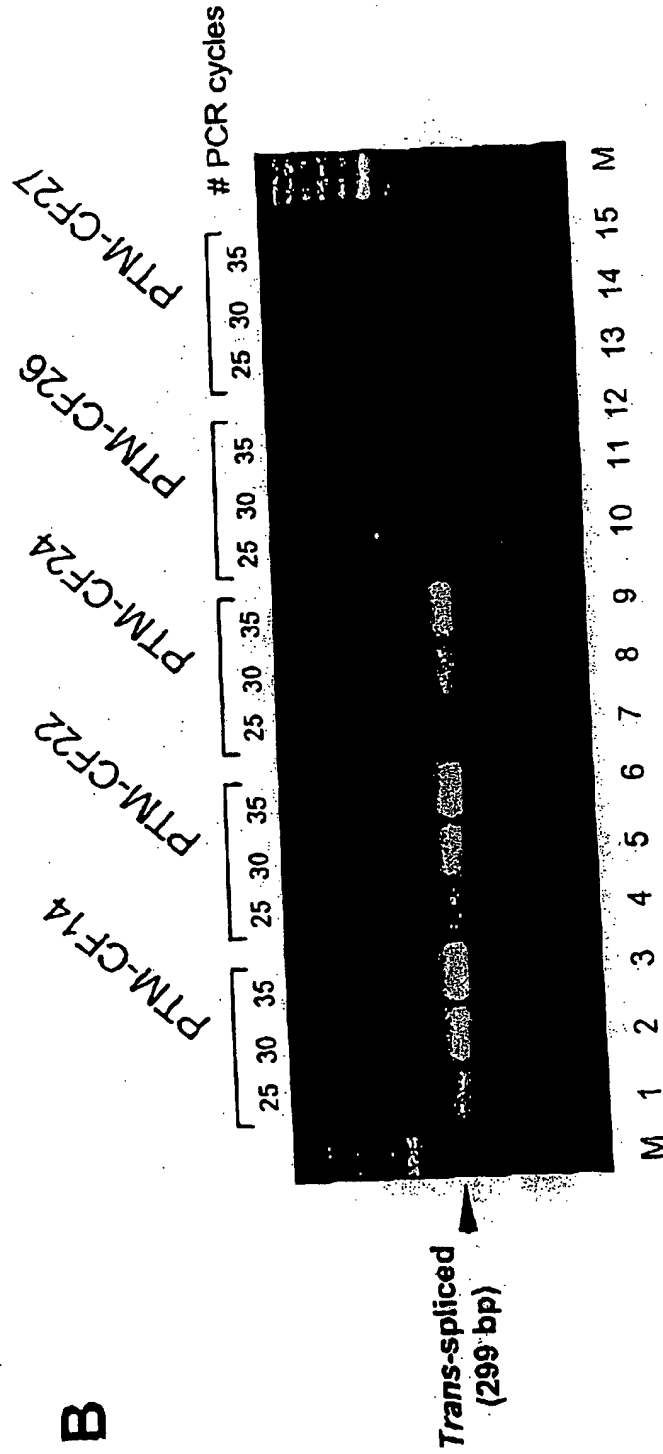


Figure 4B

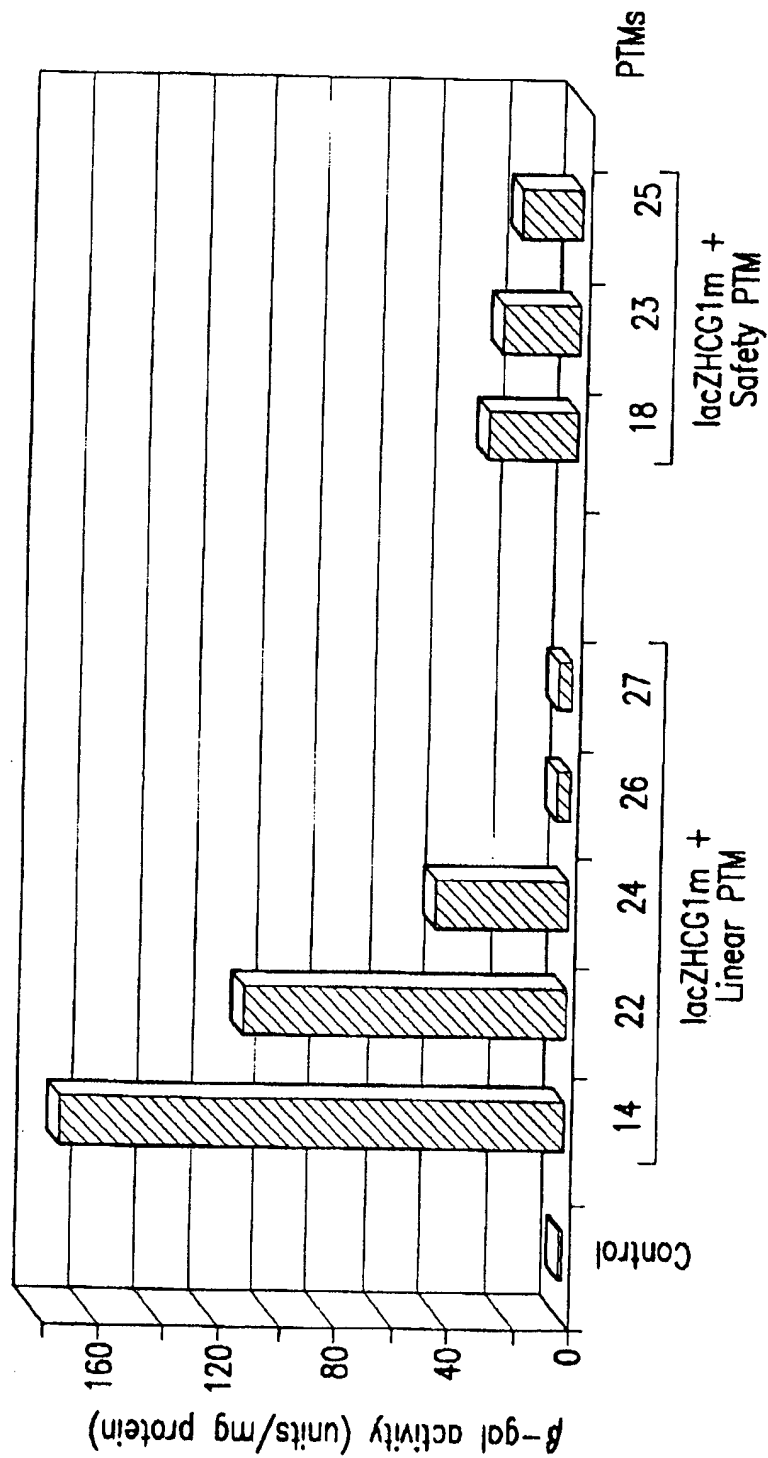


FIG.41C

Exons
1-10

ATGCAGAGGTCGCCTCTGGAAAAGCCAGCGTTGTCTCCAACTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
 GATACAGACAGCGCCTGGAATTGTCAGACATATACCAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
 CGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTATGCCCTTCGGCGATGTTTTTCTGG
 AGATTTATGTTCTATGGAATCTTTTATATTTAGGGGAAGTCACCAAGCAGTACAGCCTCTCTTACTCGGAAGAATCA
 TAGCTTCTATGACCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGCCTTATGCCTTCTCTTTAT
 TGTGAGGACACTGCTCTACACCCAGCCATTTTGGCCTTCATCACATGGAATCCAGATGAGAATAGCTATGTTTAGT
 TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTTGTAGTCTCCTT
 CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
 CATGGGCTAATCTGGGACTTGTACAGGCGTCTGCCCTCTGTGGACTTGGTTTCTGATAGTCTTGGCCCTTTTCAG
 GCTGGGCTAGGAGAATGATGATGAAGTACAGAGATCAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG
 AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAATGATTGAAAACCTAAGACA
 AACAGAACTGAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTCTCAGGGTCTTT
 GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGAAAAATATTACCACCATCTCATTCT
 GCATTGTTCTGCGCATGGCGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA
 CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATACTTAACGACTACAGAAGTAGTGATGGAG
 AATGTAACAGCCTTCTGGGAGGAGGATTGGGGAATTATTGAGAAAGCAAAACAAACAATAACAATAGAAAACTT
 CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGCTGAAAGATTAATTTCAGAT
 AGAAAGAGGACAGTTGTTGGCGTTCCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGCGAG
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGCCCGCATCAGCTTTGCAGCCAATTCAGTTGGATCATGCCCGTA
CCATCAAGGAGAACAATAATCTTCGGGTCACTTACGACAGTACCGCTATCCCTCGGTGATTAAAGCCCTGTCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTCCGGCCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTGGAAAAGTATAACACAATGAAATTTCTTCCACTGT
GCTTAATTTTACCCTCTGAATTTCTCCATAATCATATTACAACCTGAACCTCGGAAATAAAACCATCATT
ATTAACCTATTATCAAAATCACGCT

FIG.42

153 bp PTM24 Binding Domain:

Nhe I

153 bp BD underlined

GCTAGC-AAATAATGACGAAGCGCCCTCAGGCTCAGGATTCACCTGCTCCCAATTATCAATCCTAAGCAGAAGTGTATA

TTCATTGTAAGATTCTATTAACTCAATTGATTCAAAATATTAAATACTTCCTGTTTACCTACTCTGCTATGC

Sac II

AC-CCGCGG

FIG.43A

Trans-splicing domain

AATAATGACGAAGCCGCCCTCAGGCTCAGGATTCACCTTGGCCCTCCAATTATCATCCTAAGCAGAAGTGTATATCTTA
TTTGTAAGATTCTATTAACCTCATTGATTCAAATATTTAAATACTTCCTGTTTCACCTACTCTGCTATGCCCCGC
GGAACATTATTATAACGTTGCTCGAATACTAAGTGGTACCTCTCTTTTTTTTGATATCCTGCAG

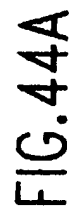
Exons 10-24

ACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGAAGAATTTCATCT
 GTTCTCAGTTTTCTGGATTATGCCCTGCCACCATTAAAGAAAATATCATCTTTGGTGTTCCTATGATGAATATAGATA
 CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTCAGAGAAAGACAATATAGTTCTTGGAGAA
 GGTGGAATCACACTGAGTGGAGGTCACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTGTATT
 TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAACCTGATGGC
 TAACAAAACCTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTCCATGAAGGT
 AGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAACTCATGGGATGTGATT
 CTTTCGACCAATTTAGTGCAGAAAGAAGAAATCAATCCTAAGTACAGCTTACACCGTTTCTCATTAGAAGGAGATGC
 TCCTGTCTCCTGGACAGAAACAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGAAAAAGGAAGATTCTATT
 CTCAATCCAATCAACTCTATACGAAAATTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT
 CTGATGAGCCTTTAGAGAGAAGGCTGTCTTAGTACCAGATTCTGAGCAGGAGAGGGGATACCTCGCATCAGCGT
 GATCAGCACTGGCCCAAGCTTCAGGCACGAAGGAGGAGTCTGTCTGAACCTGATGACACACTCAGTTAACCAAGGT
 CAGAACATTCACCGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCTCAGGCAAACTGACTGAACCTGGATA
 TATATTCAAGAAGGTTATCTCAAGAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT
 TTTTGATGATATGGAGAGCATACCAGCAGTACTACATGGAACATACCTTCGATATATTACTGTCCACAAGAGCTTA
 ATTTTGTGCTAATTTGGTGCTTAGTAATTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTCTGTGCTCCTTGGAA
 AACTCCTCTTCAAGACAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTT
 GTATTATGTGTTTTACATTACGTGGGAGTAGCCGACACTTTGCTTGTATGGGATTCTTCAGAGGTCTACCAGTGGT
 CATACTCTAATCAGAGTGTGAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCTCA
 ACACGTTGAAAGCAGGTGGGATTCTTAATAGATTCTCCAAGATATAGCAATTTTGGATGACCTTCTGCCCTCTTACCAT
 ATTTGACTTCATCCAGTTGTTATTAATTTGATTGGAGCTATAGCAGTTGTGCGAGTTTTACAACCTACATCTTTGTT
 GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCTCCAACCTCACAGCAACTCAAACAACCTGG
 AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTACAAGCTTAAAGGACTATGGACACTTCGTGCCCTTCGGACC
 GCAGCCTTACTTTGAAACTCTGTTCACAAAGCTCTGAATTTACATACTGCCAACTGGTCTTGTACCTGTCAACACTG
 CGCTGGTTCCAAATGAGAATAGAAATGATTTTGTCACTTCTTTCATTGCTGTTACCTTCATTTCCATTTTAAACAACAG
 GAGAAGGAGAAGGAAGAGTTGGTATTATCTGACTTAGCCATGAATATCATGAGTACATTGCAGTGGCTGTAAACTC
 CAGCATAGATGTGGATAGCTTGATGGATCTGTGAGCCGAGTCTTTAAGTTCAATTGACATGCCAACAGAAGGTAAACCT
 ACCAAGTCAACCAACCATAACAAGATGGCCAACCTCTCGAAAGTTATGATTATTGAGAATTCACAGGTGAAGAAAGATG
 ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAATGCCATATTAGA
 GAACATTTCTTCTCAATAAGTCTGGCCAGAGGTGGGCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTA
 TCAGCTTTTTTGGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTCTTGGGATTCAATAACTTTGCAAC
 AGTGGAGGAAAGCCTTTGGAGTGATACCACAGAAAGTATTTATTTTTCTGGAACATTAGAAAAAACTGGATCCCTA
 TGAACAGTGGAGTGATCAAGAAATATGGAAGTTGCAGATGAGGTGGGCTCAGATCTGTGATAGAACAGTTTCTGGG
 AAGCTTGACTTTGTCTTGTGGATGGGGCTGTGTCTTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
 TTCTCAGTAAGGCGAAGATCTTGTGCTTGATGAACCCAGTGCCTATTGGATCCAGTAACATACCAATAATTACAAG
 AACTCTAAAACAAGCATTGTCTGATTGCACAGTAATTTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
 TTTTTGCTCATAGAAGAGAACAAGTGGCGCAGTACGATTCCATCCAGAACTGCTGAACGAGAGGAGCCTCTTCCGGC
 AAGCCATCAGCCCTCCGACAGGGTGAAGCTCTTTCCCAACCGAACTCAAGCAAGTGAAGCTTAAGCCCCAGATTGC

Histidine log Stop

TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATTAG

FIG.43B



204040" 2544660

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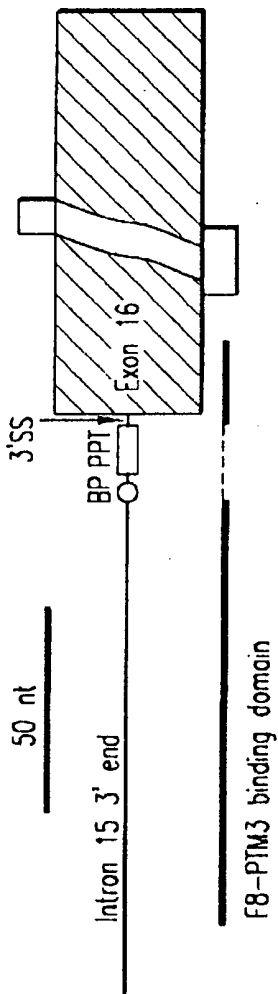


FIG.44B

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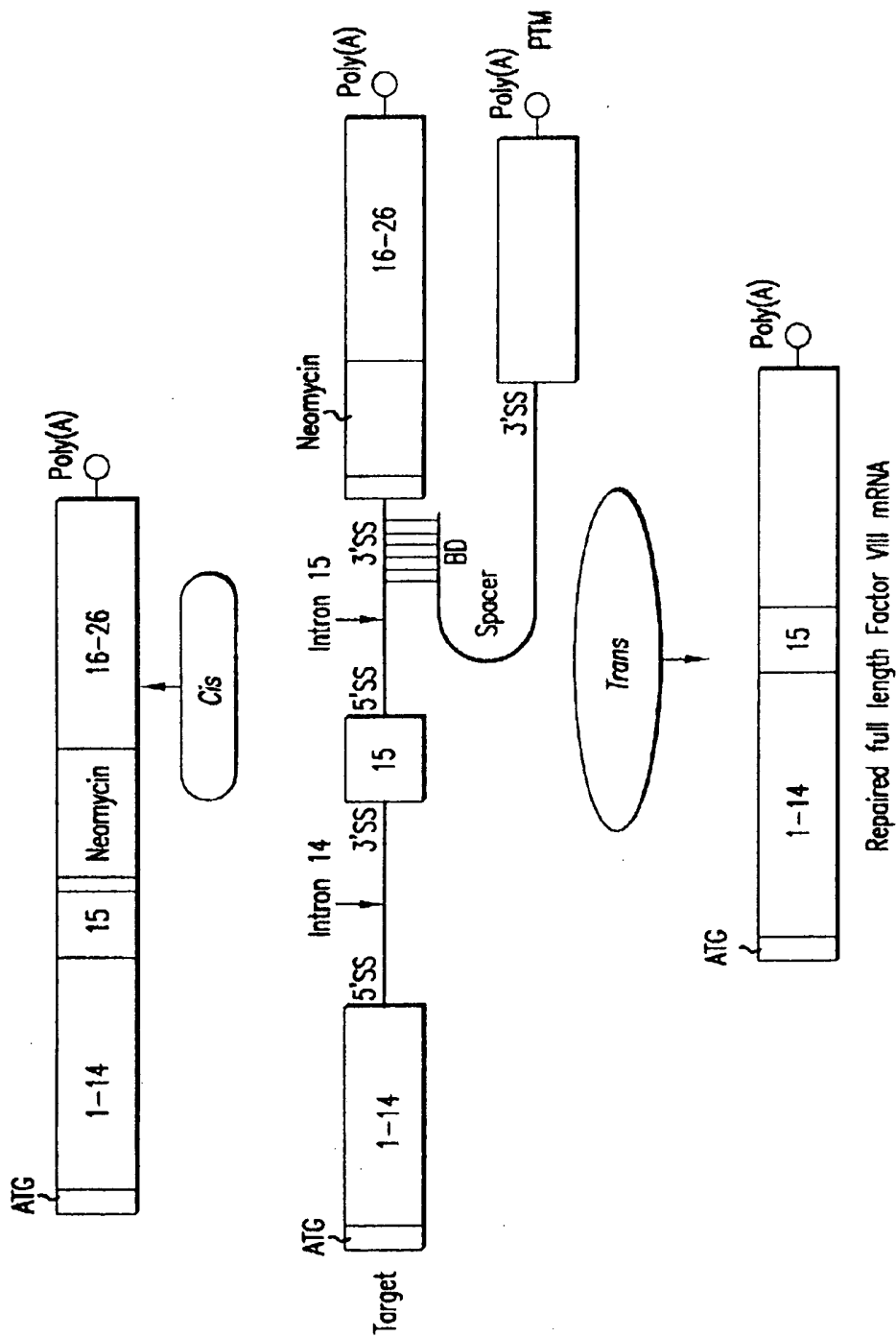
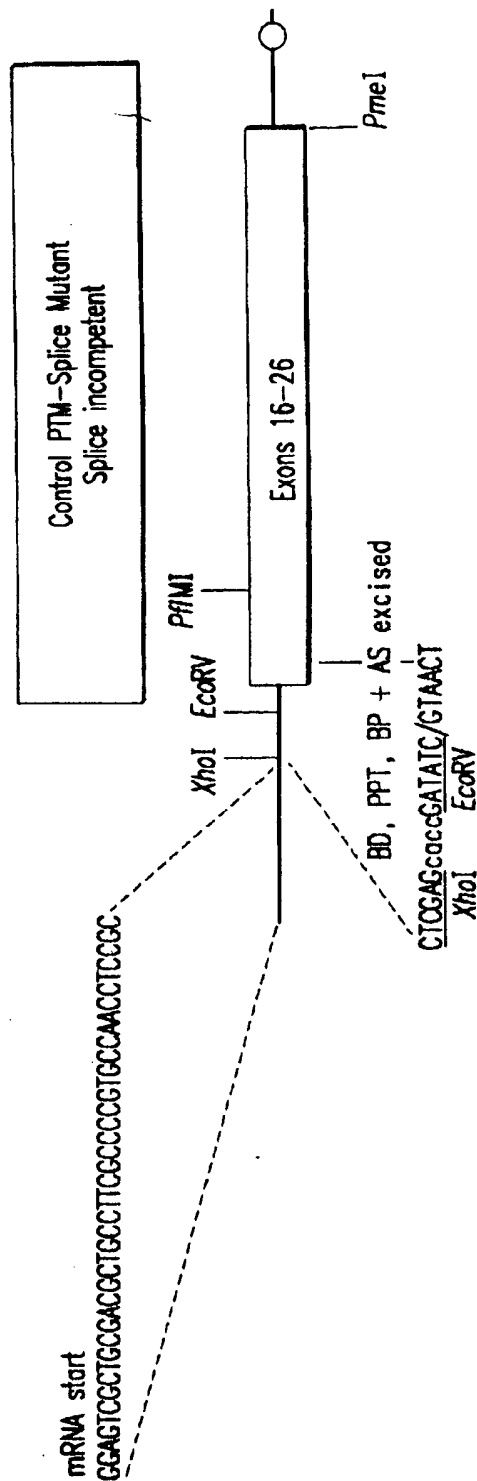


FIG.44D

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Method:
Excise TSD and part of exon 16 with
XhoI and PflMI and ligate in a PCR product that:
1) eliminates the TSD and splice acceptor site
2) inserts EcoRV adjacent to exon 16
3) restores the coding for exon 16

FIG.45

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Repair of Factor VIII
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice
after IV PTM-FVII intraportal infusion
(100 μ gDNA)(n=3)

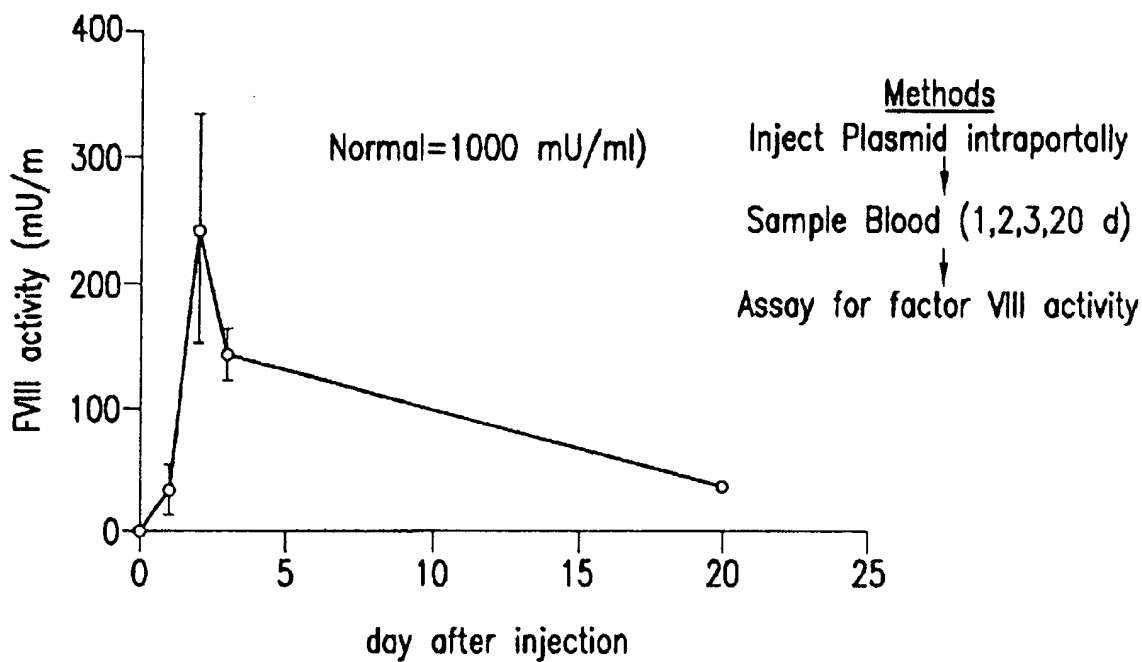


FIG.46

201040" 26474660

Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

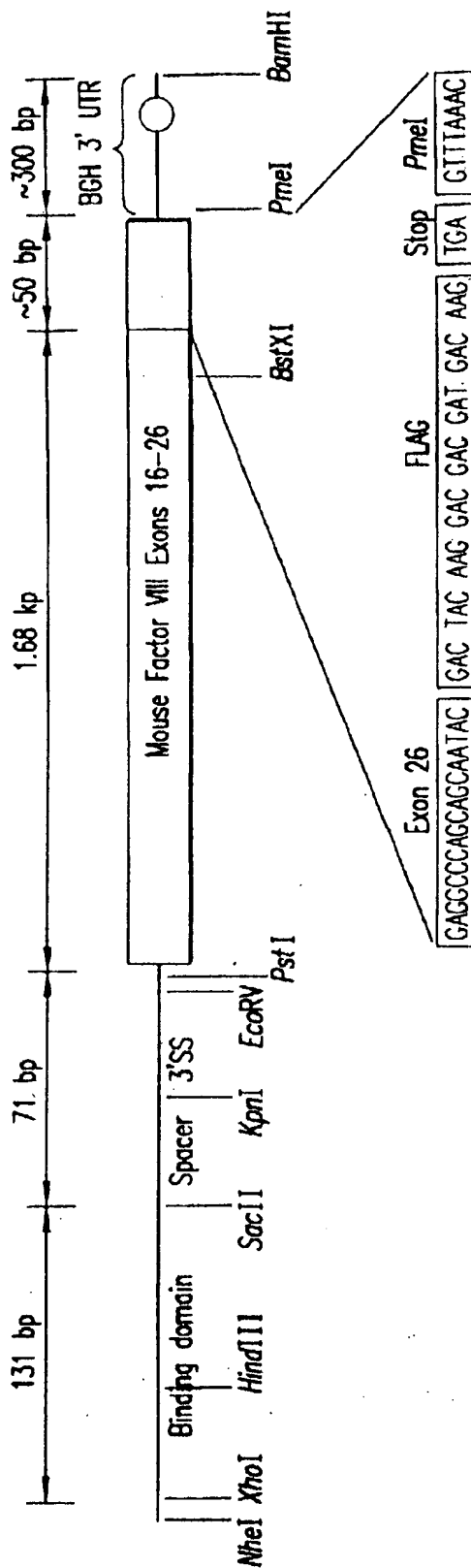
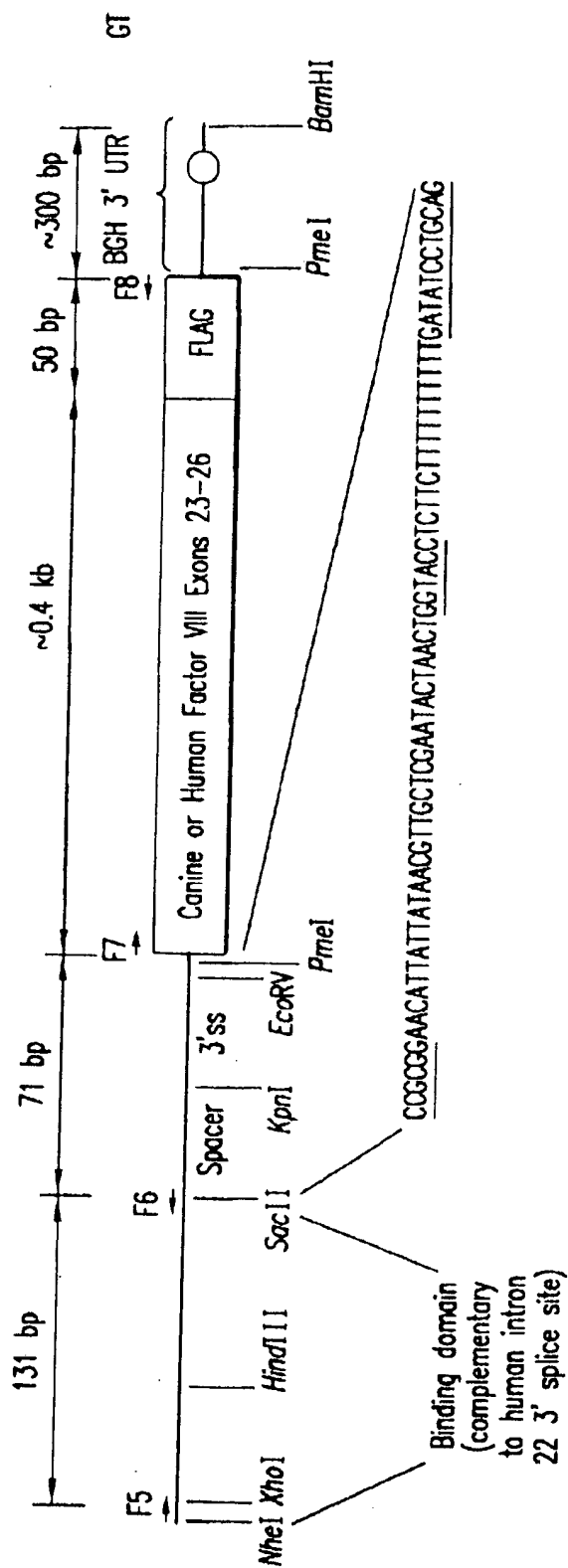


FIG.47A



FLAG=C-terminal tag to be used to detect repaired factor VIII protein.

FIG. 47B

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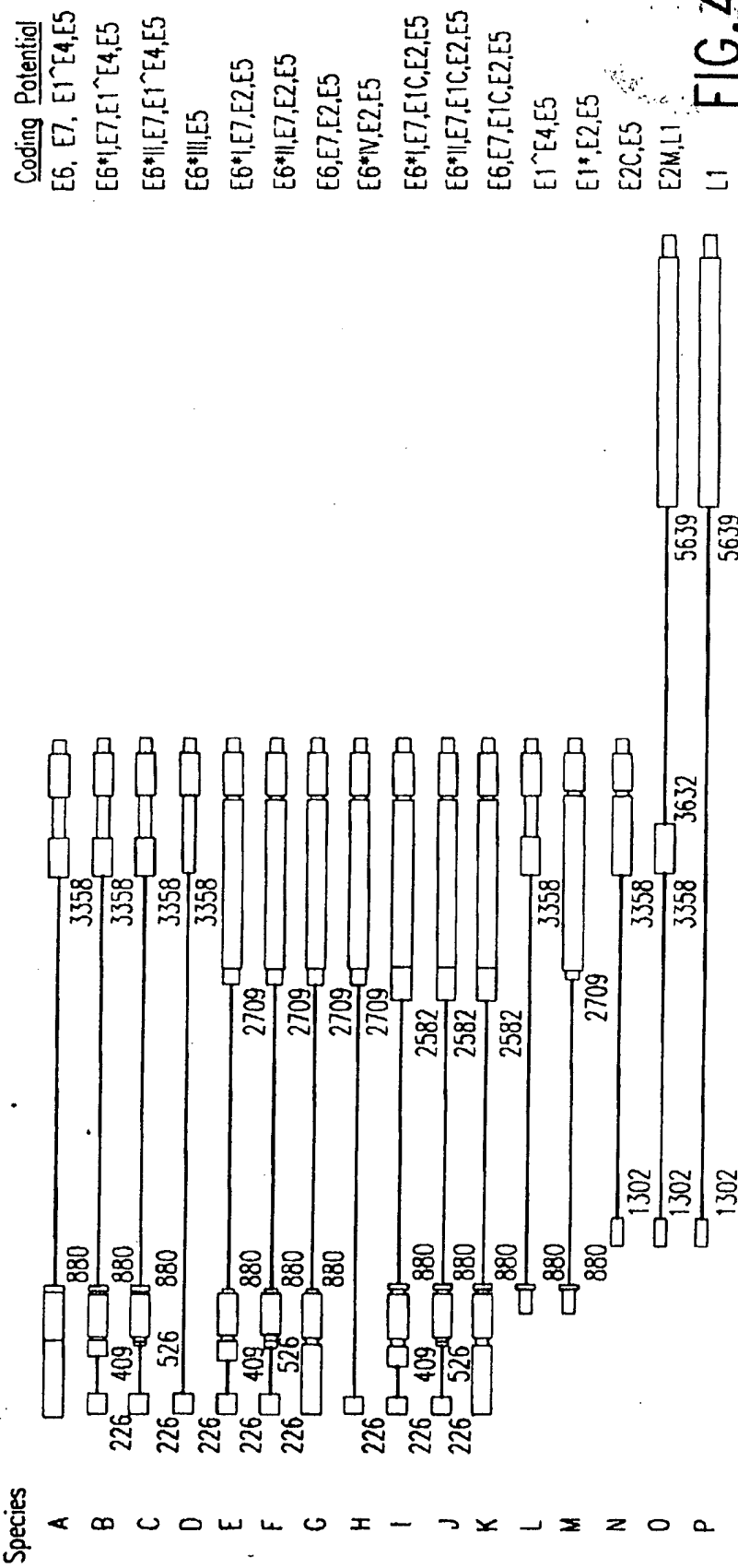
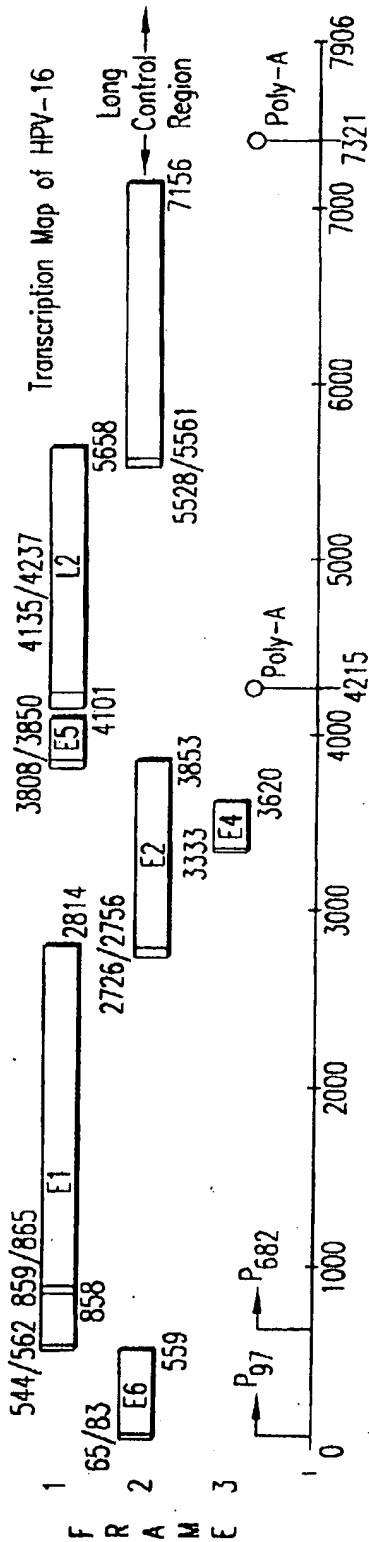
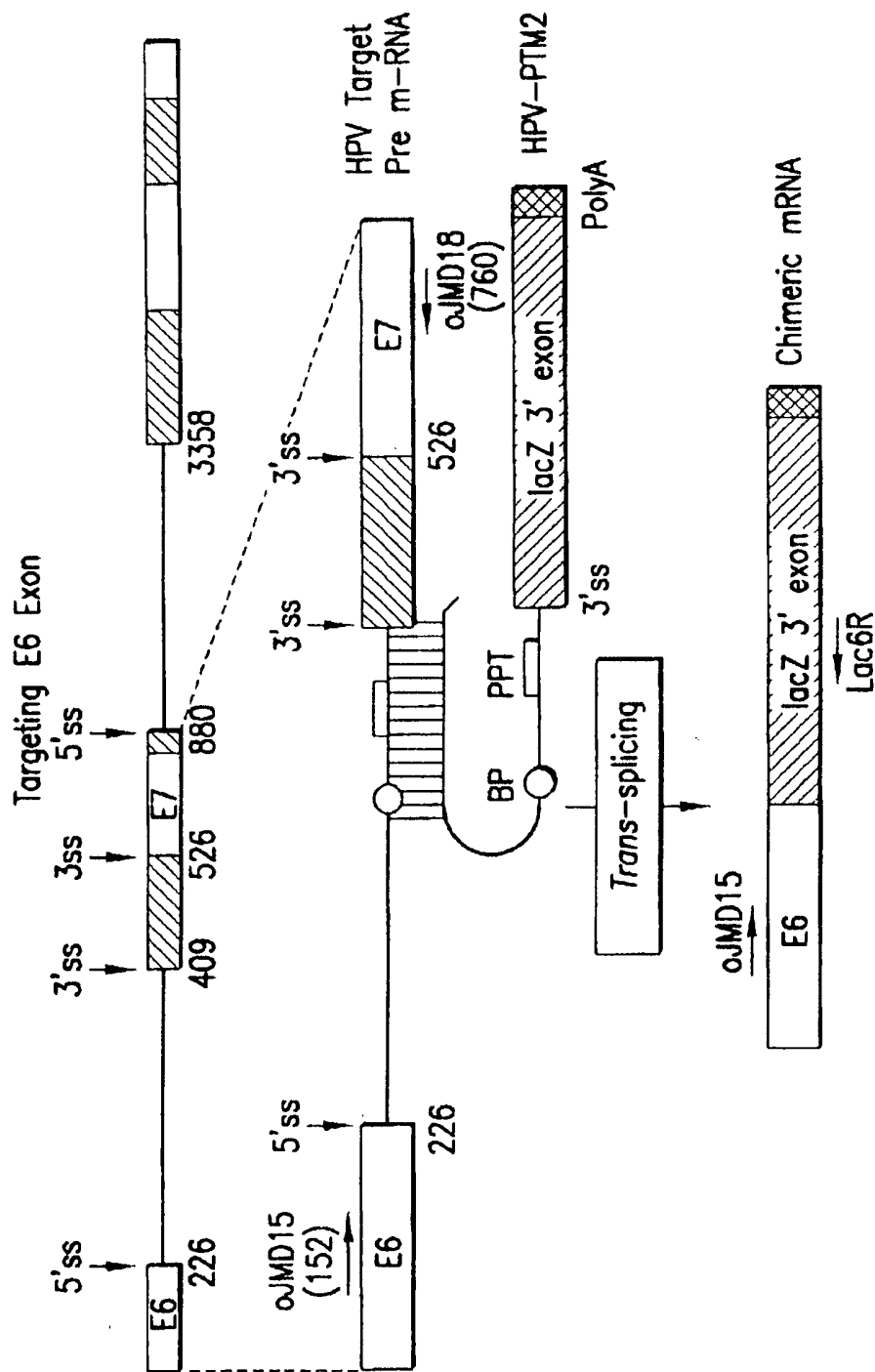


FIG. 48

SMART Strategy to Disrupt the Expression of Human Papillomavirus Type 16



SMART Strategy by 3' Exon Replacement: Schematic diagram of HPV-PTM2 binding to the 3' splice site of the HPV type 16 target pre-mRNA

FIG.49

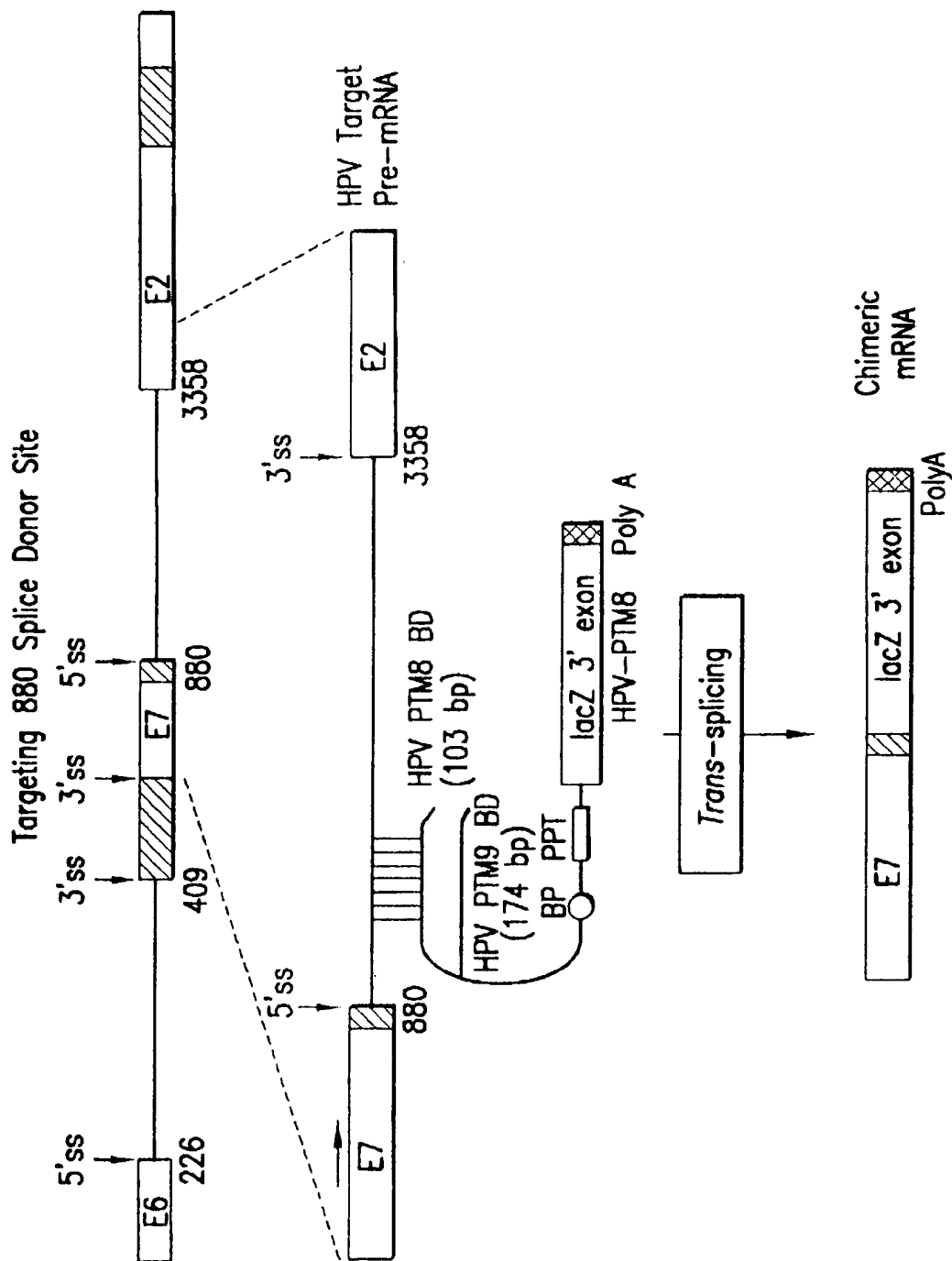


FIG.50

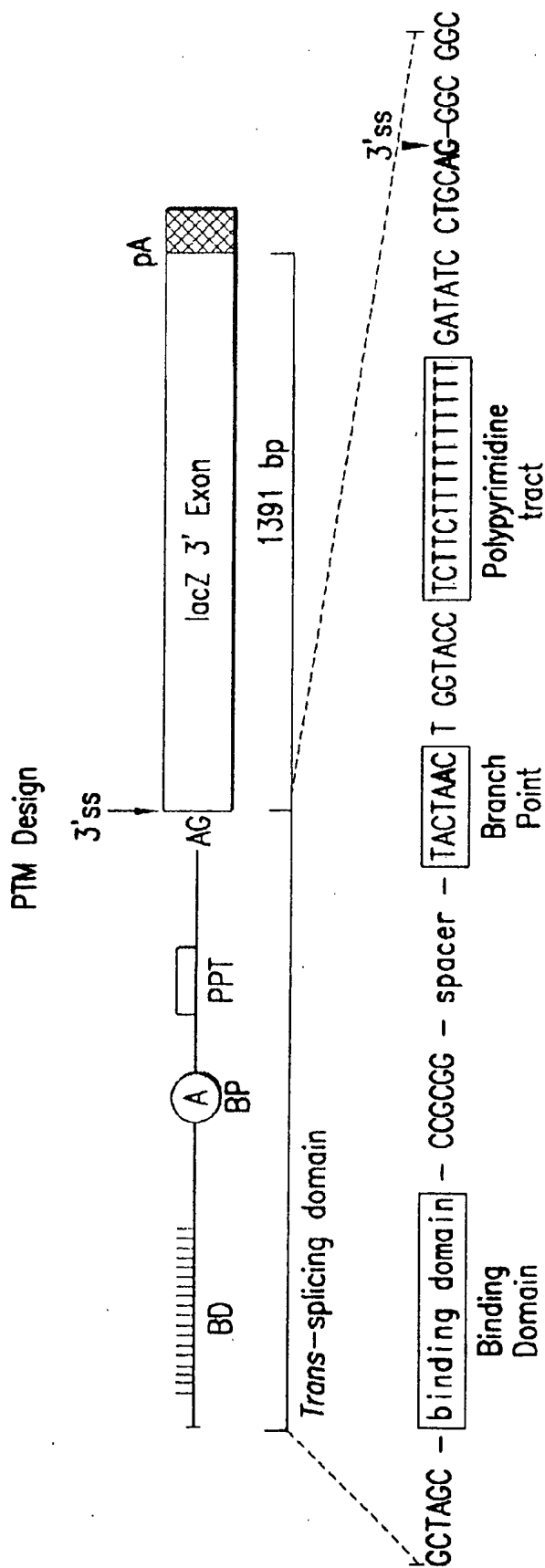
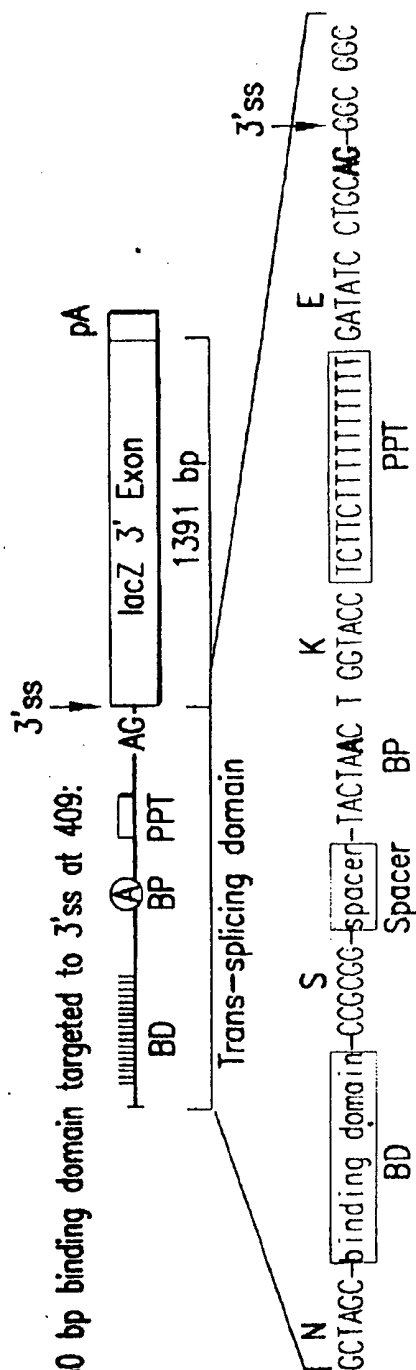


FIG.51

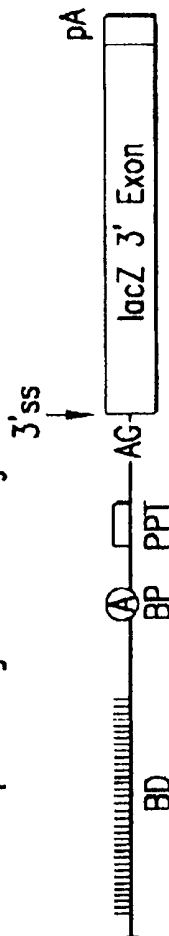
HPV-PTM1 with 80 bp binding domain targeted to 3' ss at 409:



Binding domain sequence: CAGTTAAATAC ACCTAAATTAA CAAATCACAC AACGCTTTGT TGTATTGCTG
TTCCTAATGTT GTCCCATACA CACTATAACA

FIG. 52A

HPV-PTM2 with 149 bp binding domain targeted to 3' ss at 409:



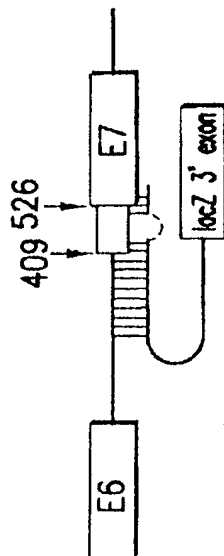
Binding domain sequence: CAGTTAATAC ACCTAAATTAA CAAATCACAC AACGCTTTGT TGTATTGCTG
TTC7AATGTT GTTCCATACA CACTATAACA ATAATGTCTA TACTCACTAA
TTTTAGAA7A AACCTTTAA CATTATATCAC ATACAGGATA TCGATTCCCC

FIG. 52B

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Binding Domains of HPV-PTM3 and 4

HPV-PTM3 Binding domain (covers both 3'ss at 409 and 526; has 53 bp bubble)
 GATGATCTGCAACAAGACATACATCGACCGGTCCA(53 nt bubble)CTTCAGGACACAGTGGCTTTTGAC
 AGTTAATACACCTAATTAAACAATCACACAACGGTTTGTGTTATTCAGTTCCTAATGTTGTTCCATACACACTA
 TAACAAT



HPV-PTM4 Binding domain (covers both 3'ss at 409 and 526; has 76 bp bubble)

GATGATCTGCAACAAGAC(76 nt bubble)GACACAGTGGCTTTTGACAGTTAATACACCTAATTACAAATC
 ACACAACGGTTTGTGTTATTCAGTTCCTAATGTTGTTCCATACACACTATAACAAT

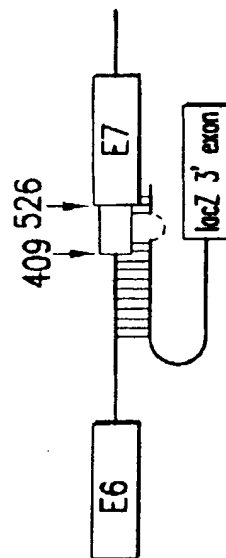
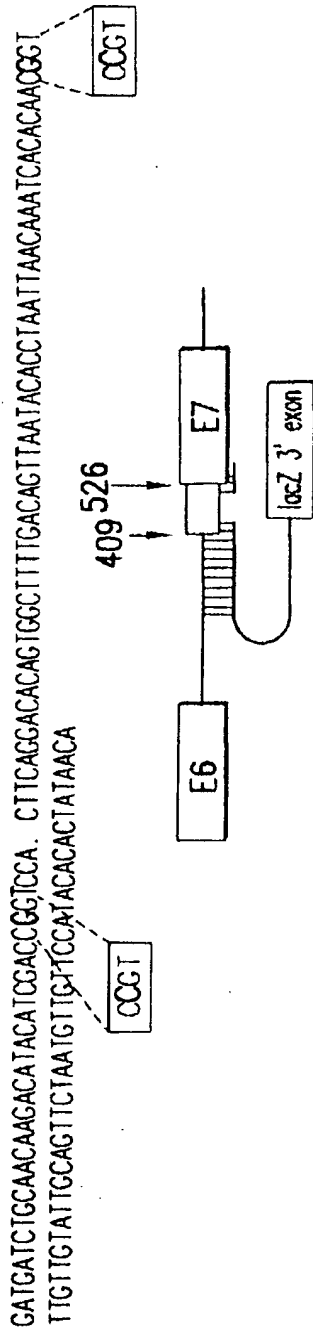


FIG.53

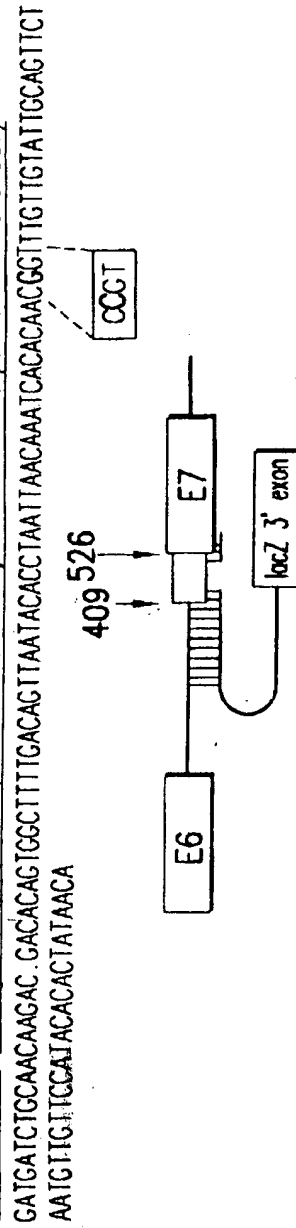
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HPV-PTM5 and 6

HPV-PTM5, Binding domain (140 nt, has 53 nt bubble, covers 3'ss at position 409 and 526)

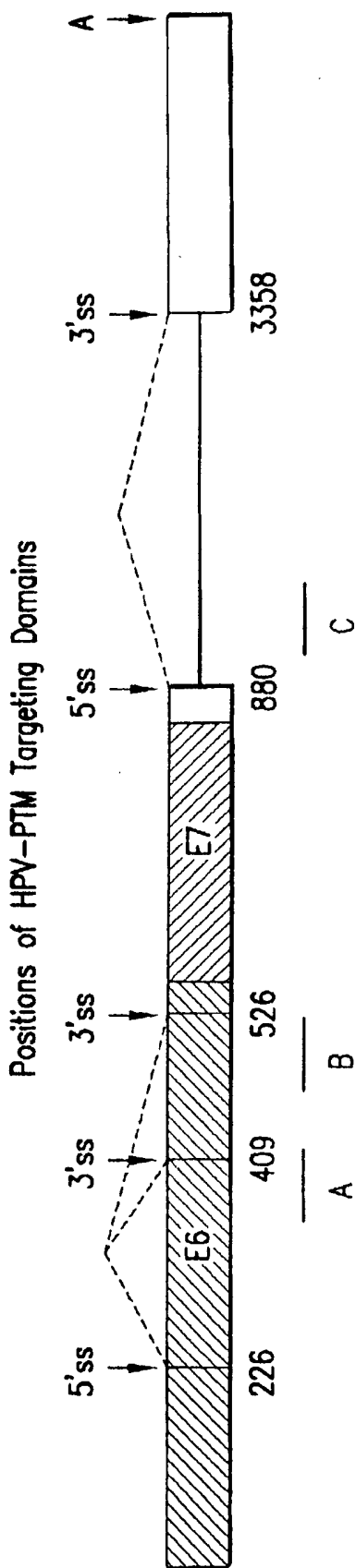


HPV-PTM6, Binding domain (117 nt, has 76 nt bubble, covers 3'ss at position 409 and 526)



Note: Nucleotides in bold are modified to prevent PTMs cryptic splicing

FIG.54

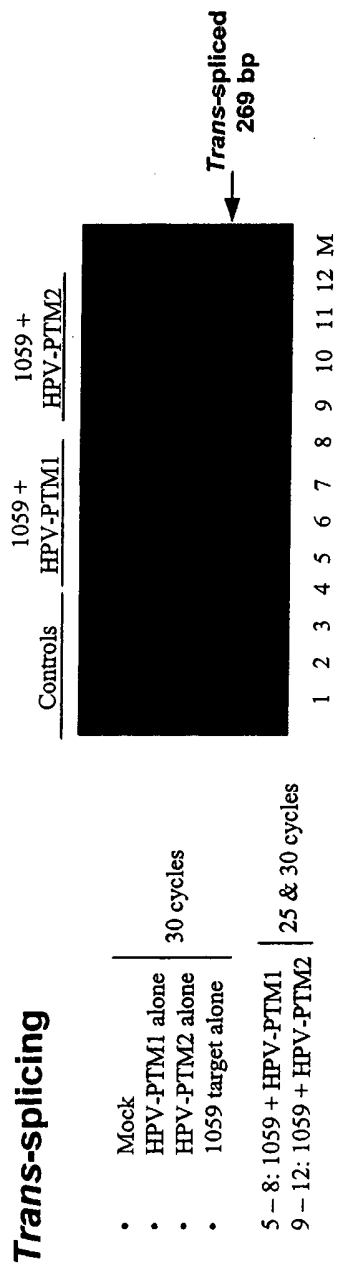
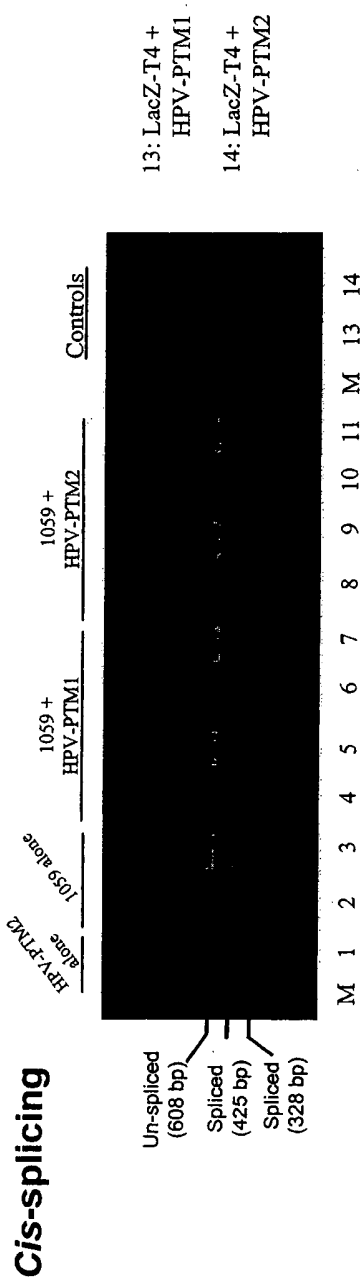


PTM	Region	Size (nt)
HPV-PTM1	A	80
HPV-PTM2	A	149
HPV-PTM5	A+B	140
HPV-PTM6	A+B	117
HPV-PTM8	C	104
HPV-PTM9	C	174

FIG.55

80 2 91

Trans-splicing Efficiency of HPV-PTMs in 293T Cells



RT-PCR Analysis of total RNA

INIRON

FIG. 56

Trans-splicing between target pre-mRNA and
PTM is accurate (293T cells)

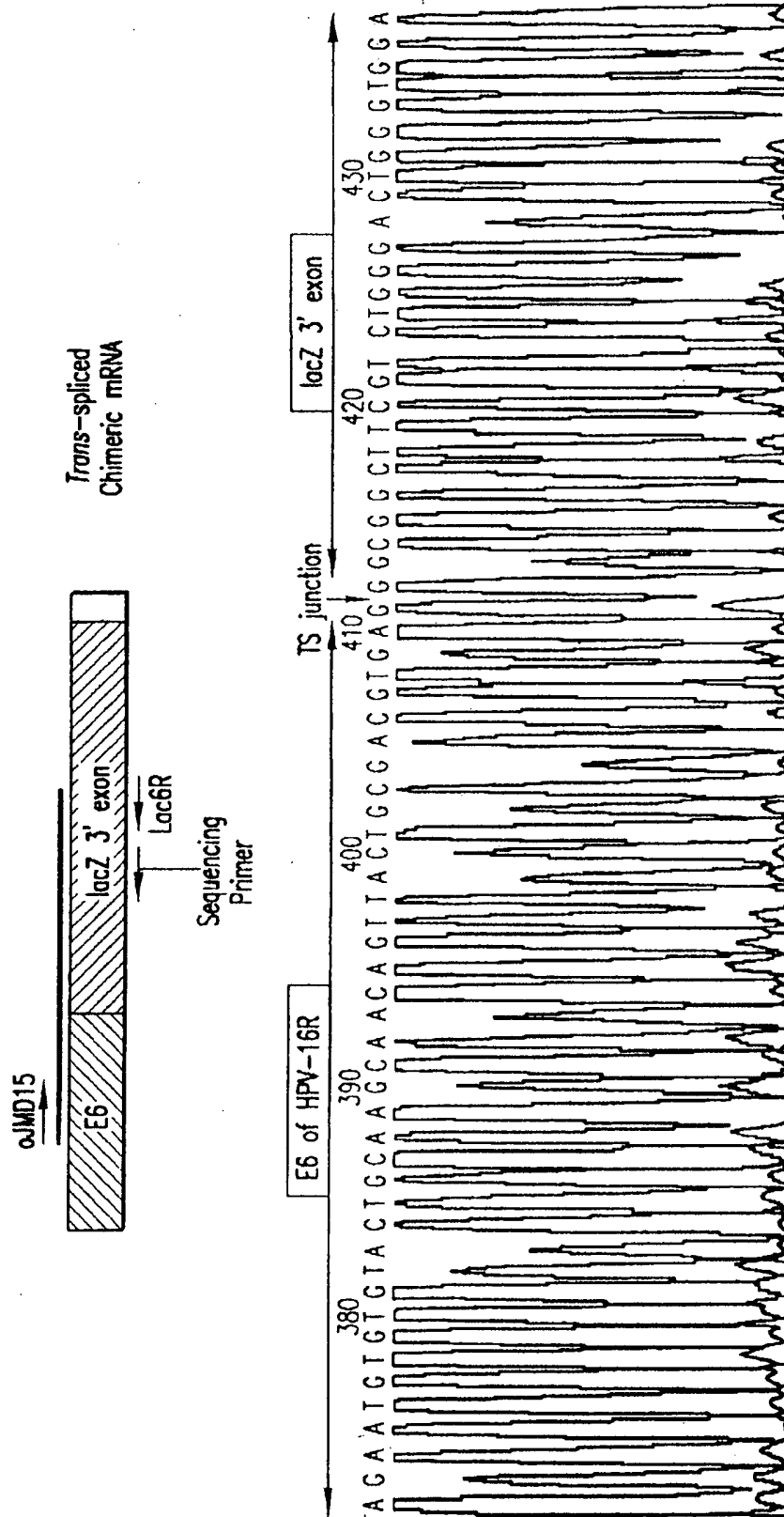
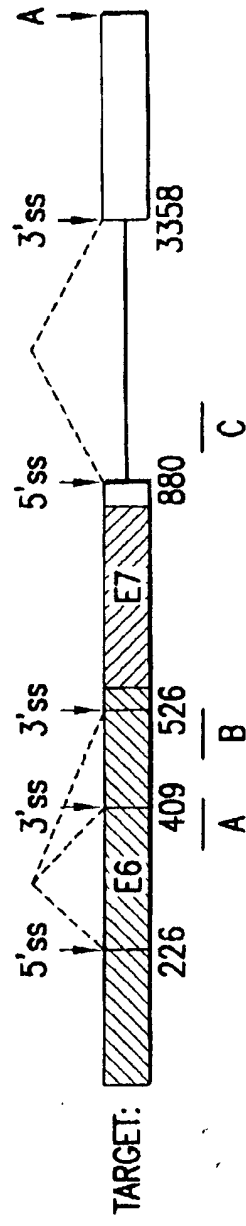


FIG. 57

Trans-splicing in 293 Cells (Co-transfections)



PTM	Binding Domain		% trans-spliced	
	Region	Size (nt)	226 sd	880 sd
HPV-PTM1	A	80	69	0.6
HPV-PTM2	A	149	45	0.9
HPV-PTM5	A+B	140	55	0.8
HPV-PTM5ΔBP/PPT	A+B	140	0.5	0.2
HPV-PTM6	A+B	117	59	1
HPV-PTM8	C	104	7	37
HPV-PTM9	C	174	14	22
CF-PTM27	CF intron	411	0	0

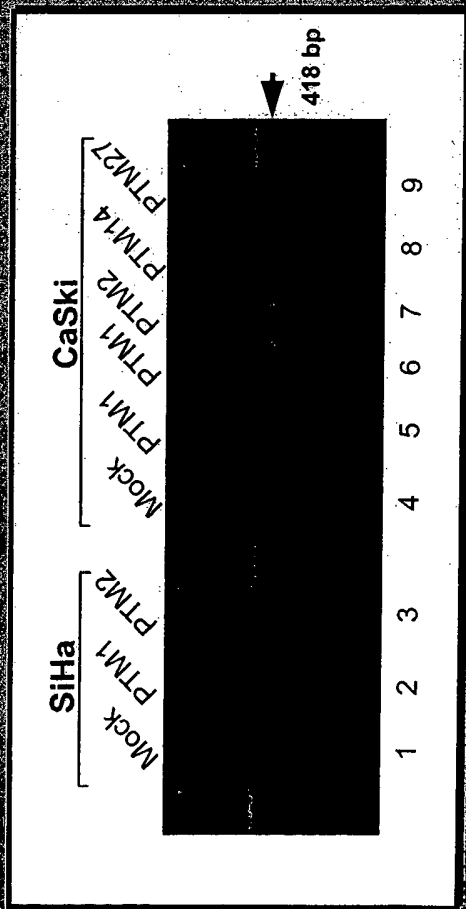
Quantification of Trans-splicing efficiency using real-time QRT-PCR

FIG.58

82 8 91

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Trans-splicing into Endogenous HPV Pre-mRNA Target in SiHa & CaSki Cells



SiHa: Single copy
CaSki: ~400-500 copy/cell



RT-PCR Analysis of total RNA

- RT-PCR Conditions**

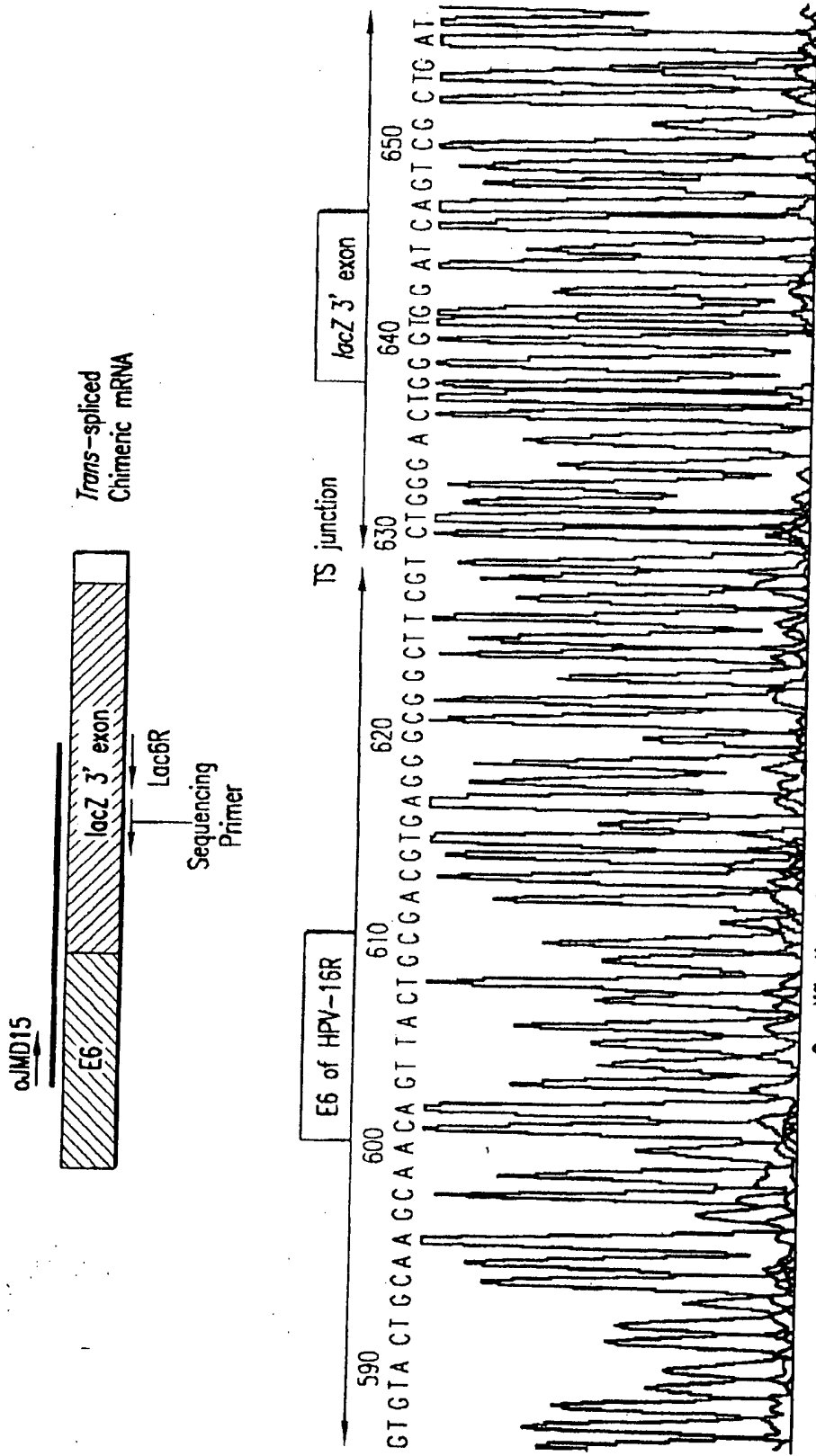
 - Total RNA: 400 ng/rxn
 - Primer's: oJMD15 + Lac16R
 - # Cycles : 35
 - Expected product : 418 bp
- Details**

 - PTM1, PTM2 : HPV targeted, specific
 - PTM14 : CF targeted, non-specific, has 23 bp BD
 - PTM14 : CF targeted, non-specific, has 411 bp BD

INTRONN

FIG. 59

Accurate Trans-splicing of HPV-PTM1
in Si Ha Cells (Endogenous target pre-mRNA)



Quantification of trans-splicing efficiency using real-time QRT-PCR

FIG.60

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Trans-splicing in SiHa Transfections
(Endogenous target)

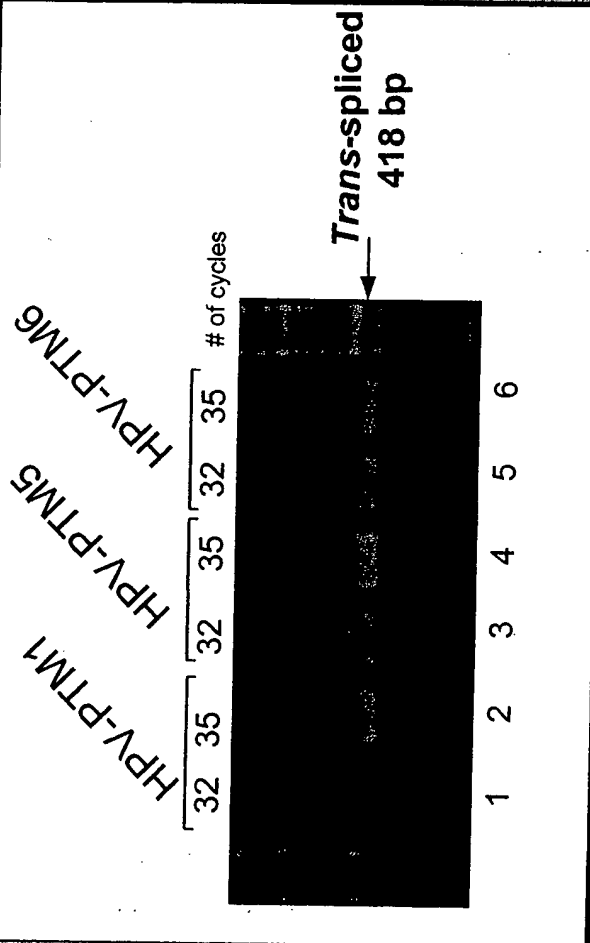
<u>PTM</u>	<u>% trans-spliced</u>
pcDNA3.1	0
HPV-PTM1	0.16
HPV-PTM5	0.12
HPV-PTM6	0.11
CF-PTM27	0

Quantification of *trans*-splicing efficiency using real-time QRT-PCR

FIG.61

09041452 040402

Trans-splicing Efficiency of HPV-PTM1, 5, & 6 in SiHa Cells

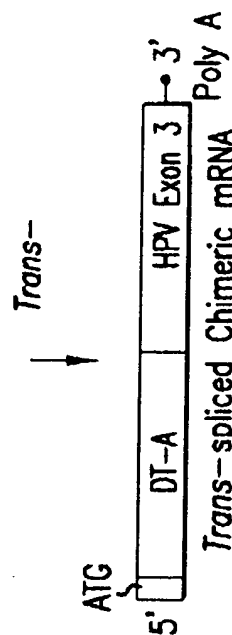
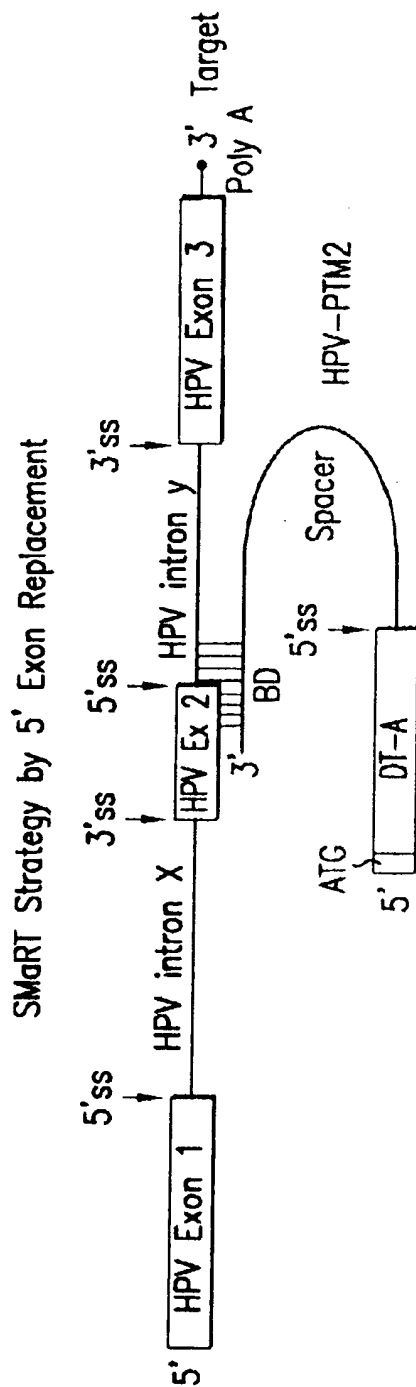


- SiHa cells transfected with 1.5 µg plasmid DNA, LipoPlus
- RNA isolated after 48 hr
- Total RNA: 500 ng/Rxn
- Primers: oJMD15 + Lac16R
- Expected product: 418 bp

1, 3, 5, : 32 cycles
2, 4, 6, : 35

INTRON

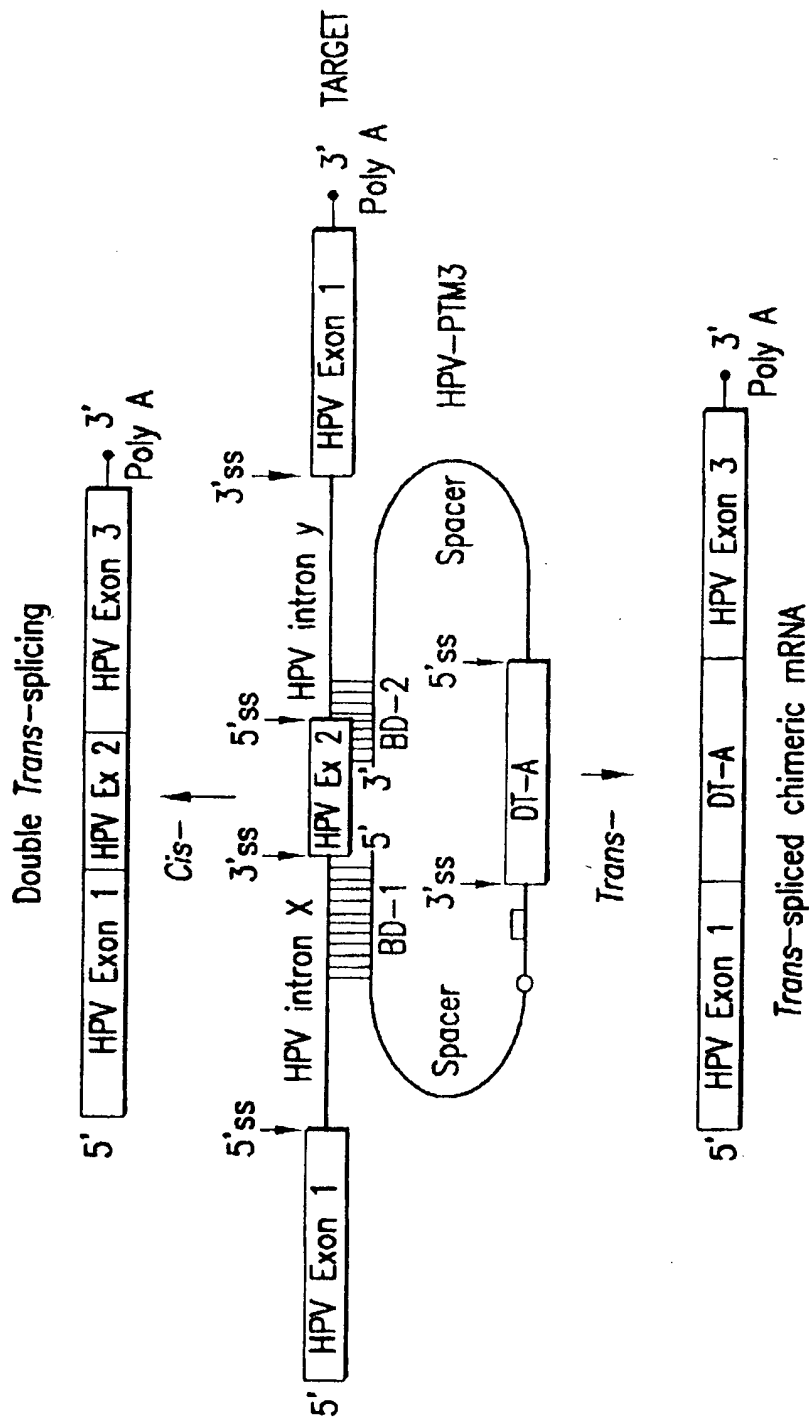
FIG. 62



Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

FIG.64

89 91



Schematic diagram of a double Trans-splicing PTM binding to the 3' and 5' splice sites of the HPV mini-gene target

FIG.65

5' HPV Exon 1 HPV Exon 2 3' Cis-spliced mRNA
Poly A

5' HPV Exon 1 5'ss GU BP PPT AG HPV Exon 2 3' Poly A TARGET
BD

Spacer BP PPT AG DT-A 3' Poly A HPV-PTM

Trans-spliced Chimeric mRNA
5' HPV Exon 1 DT-A 3' Poly A

SMaRT Strategy by 5' Exon Replacement: Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

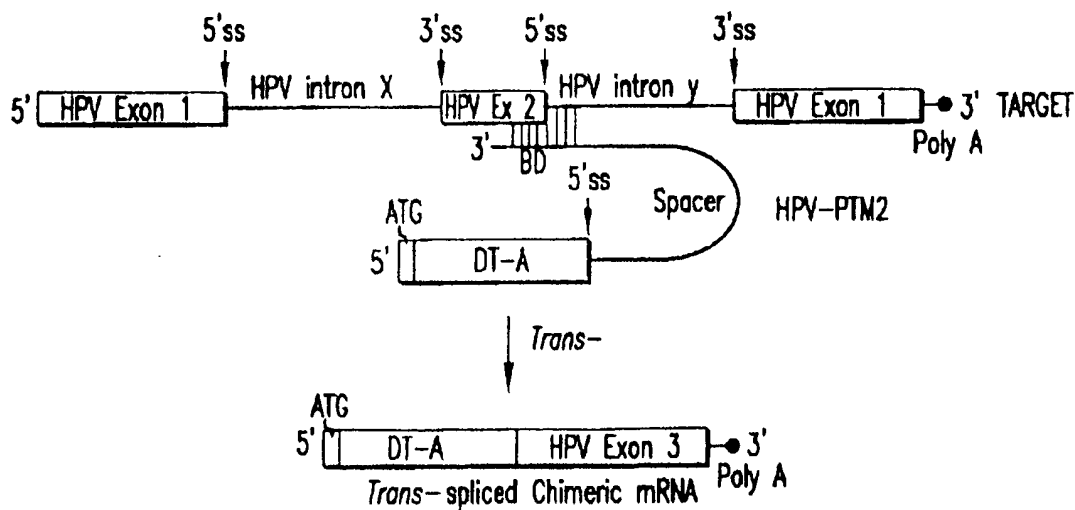


FIG. 66B

HPV-PTM3 (For Internal exon replacement)

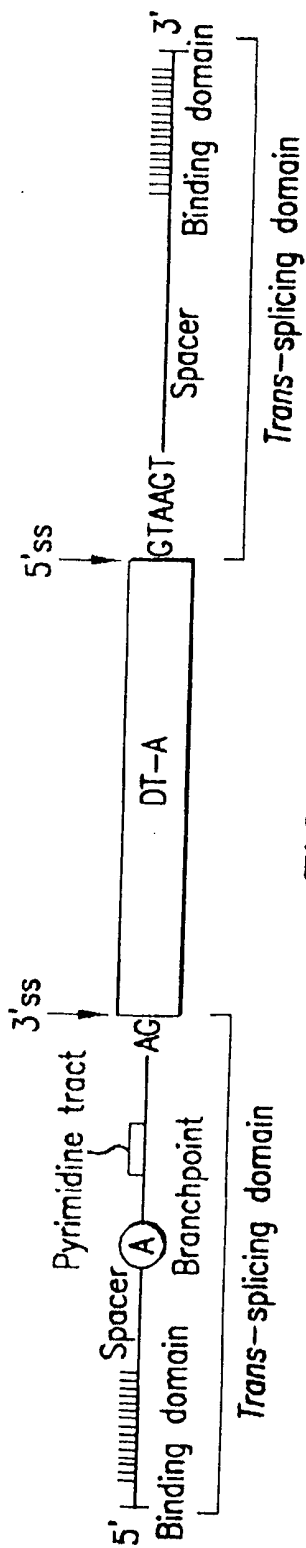


FIG.67

91 8 91